The influences of weather on patients with different ovarian responses in the treatment of assisted reproductive technology

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Purpose: The purpose of this study was to evaluate the influences of tropical weather on patients with different ovarian responses in the treatment of assisted reproductive technology.

Methods: Six-hundred forty-seven women underwent their first treatment cycles were retrospectively analyzed. Patients received embryo transfer either 3 days or 5 days after oocyte retrieval, depending on the number and quality of embryos on day-2.

Results: Significant correlations were demonstrated in the top quality embryo rates of day-3 and day-5 embryo transfers with temperature, humidity, and atmosphere pressure. The cumulative light hours negatively correlated with the implantation and pregnancy rates of day-3 embryo transfer ($-.282$ and $-.282$, respectively), while they positively correlated with those of day-5 embryo transfer ($.225$ and $.224$, respectively).

Conclusions: These results clearly suggest that weather may exert influences on the outcome of assisted reproductive technology. Patients with different ovarian responses or blastocyst culture and transfer may modify weather influences.

KEY WORDS: Blastocyst transfer; embryo; embryo transfer; pregnancy; weather.

INTRODUCTION

As outcome of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) improves, correlating factors other than laboratory procedures have been investigated. Scientists and epidemiologists have previously reported various IVF/ICSI outcomes in correlation with or without seasonal and weather influences in geological areas distant from the equator such as Canada, Austria, Israel, and United

Kingdom (1–5). Correlations of weather influence on the IVF/ICSI outcome have never been published from a tropical country. Most of the previous researches correlate seasonal or monthly variability with the general IVF-ET population. However, patients with better ovarian response in the treatment of assisted reproductive technology (ART) may behave differently to weather change than those with worse ovarian response. In our program, patients with ≥ 3 good quality four-cell embryos on day-2 after oocyte retrieval had embryo transfer on day-5. Patients with < 3 good quality four-cell embryos on day-2 had embryo transfer on the next day, fearing of diminished embryo survival if cultured until day-5. It is therefore may be of interest to look at the two groups separately. In this study, day-3 and day-5 embryo transfer cycles were analyzed and correlated with the weather parameters separately. To our

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knowledge, this is the first study to investigate the influences of tropical weather on the patients with different ovarian responses in the treatment of ART.

MATERIALS AND METHODS

Patients

Between January 1999 and December 2002, 1060 cycles were enrolled in our center, located in Southern Taiwan, for IVF/ICSI treatment. Thirty-nine cycles were cancelled due to the following reasons: low ovarian response (29), pregnant (3), vaginal spotting (1), personal reasons (6). Oocyte retrievals were performed in 1021 cycles of which only 973 cycles received embryo transfer. The 48 cycles without embryo transfer includes: no oocytes retrieved, arrest of maturation of oocytes, abnormal fertilization, no fertilization and elective embryo-frozen due to personal reasons or possible consequences for ovarian hyperstimulation syndrome (OHSS). Two-hundred and fifty-one short protocol ovarian stimulation cycles were further excluded, only long-protocol ovarian stimulation cycles were enrolled in this study. Finally, 647 patients underwent their first treatment cycle, were included with 325 day-3 embryo transfers (D3ET) and 322 day-5 embryo transfers (D5ET). The D5ET group is considered as the good outcome group, whereas the D3ET group is considered as the poor outcome group. Intracytoplasmic sperm injection was indicated when severe oligoastheno-teratozoospermia was present or in case of azoospermia whose spermatozoa was acquired from the testis (6). Data were collected and analyzed in months according to the day ova retrieval was performed. All couples completed the standard infertility workup, which included hysterosalpingography and/or laparoscopy and hormone assays in females and semen analysis in males. Patients were unselected for age, sperm parameters or infertility criteria. A computerized database maintained by the ART unit was used as well as the charts of each cycle.

Controlled Ovarian Hyperstimulation

The protocol for controlled ovarian hyperstimulation followed the standard down-regulation regimen we published previously (7). Pituitary down-regulation was evaluated by a determination of serum estradiol (E_2) concentration and a transvaginal sonography (TVS) of the ovaries. If the serum E_2

level was less than 35 pg/mL and no follicles greater than 10 mm in diameter were noted on TVS, Luprolide acetate (LA, Takeda Pharma GmbH, Stolberg, Germany) was decreased to half a dose and continued until and including the day of human chorionic gonadotrophin (hCG, N.V. Organon, Oss, Holland) administration. Serum E_2 was assayed using a commercially available competitive immunoassay with the IMMULITE Analyzer (DPC Coat-a Count; Diagnostic Products Corp., Los Angeles, CA).

If the pituitary was not suppressed, LA was continued at the same dose and the serum E_2 level was rechecked every 3 days until suppression was achieved. Patients received one of the following fixed starting doses (age <35: 225 IU/day; age $\geq$ 35: 300 IU/day) of rFSH (Gonal-F, 75 IU/ampoule, Serono, Aubonne, Switzerland) after pituitary suppression with LA. Gonadotrophin was administered daily for 5 days, after which the dose was individualized according to ovarian follicular growth. Monitoring occurred every 2 to 3 days starting on the 5th day of stimulation with TVS and serum E_2 . Human chorionic gonadotrophin (hCG, Pregnyl, N.V. Organon, Oss, Holland) 10,000 IU was administered intramuscularly when at least two follicles were 17 mm or greater in diameter with adequate serum (E_2) levels. Oocytes were retrieved 34–36 h after hCG administration.

Oocyte Preparation

For IVF, the retrieved oocyte-corona-cumulus complexes were immediately classified according to their maturity. The oocyte-corona-cumulus complexes were cultured in M2 culture medium (Medicult, Copenhagen, Denmark) for 3–8 h before insemination. For intracytoplasmic sperm injection (ICSI), the oocyte-corona-cumulus complexes were denuded and assessed shortly after retrieval. The complexes were placed in a medium with 80 IU/mL of hyaluronidase for 5 s. The cumulus and corona of the cells were removed mechanically. The denuded oocytes were cultured in an M2 culture medium for 3 to 8 h, and then were examined for the presence of the first polar body. After confirmation of the first polar body, ICSI was performed. The oocytes that did not develop to metaphase II after 8 h of incubation were discarded.

Intracytoplasmic Sperm Injection

The intracytoplasmic microinjection of a sperm into an oocyte was based on the protocol described

by Palermo *et al.* (8), except that polyvinylpyrrolidone (PVP) was eliminated from the protocol (9). The entire procedure was carried out on the heated stage of an inverted microscope.

Assessment of Fertilization, Embryo Culture, Zygote and Embryo Grading

After the IVF or ICSI procedure, oocytes were cultured as in the standard IVF procedure and assessed for the presence of pronuclei after 16–18 h of incubation. G1.2TM medium (Scandinavian IVF science) was used for the culture of embryos on days 1 to 3, and G2.2TM medium (Scandinavian IVF science) was used for the culture of embryos from day-3 to day-5. The Veeck's morphologic grading system (10) was adopted for day-2 and day-3 embryo scoring. Grade I or II embryos were defined as Top Quality Embryos (TQE). After 2 days of culture in G2.2 medium, the blastocyst formation was evaluated. The scoring assessment for the blastocyst was based on the expansion state of the blastocyst and on the consistency of the inner cell mass and trophectoderm cells (11,12). Blastocysts with grade 3, 4 or 5 AA stage are also considered as TQE (13). Quarter assisted hatching using a non-touch 1.48-micron diode laser was performed early in the morning of day-3 for all cleavage-stage embryos whether transferred or not (14). The assessment of embryo survival was based on embryo morphology and cleavage speed. Embryos that had the same number of blastomeres at two sequential times of observation, together with those zygotes that remained blocked at the pronuclear stage, were considered as developmentally arrested. A single team of embryologists coordinated all procedures, thereby ensuring that both the culture protocols and the embryo assessment were standardized.

Establishment and Follow-Up of Pregnancy

Embryos were replaced transcervically into the uterus on day-3 or 5 after oocyte retrieval, with individual consideration. We routinely offered blastocyst transfer (BT) to patients with ≥ 3 good four-cell embryos on day-2. Patients with < 3 good four-cell embryos on day-2 received day-3 embryo transfer. Luteal phase supplementation of micronized progesterone (Utrogestan, Piette International Laboratories, Belgium) 800 mg intravaginally daily, was begun on the following day of oocyte retrieval, and 5000 IU hCG was administered on the sixth day after oocyte recovery in all patients. Pregnancy was confirmed

by detecting hCG in the urine 2 weeks after transfer. Clinical pregnancy was determined by identifying a gestational sac at 7 weeks gestation by means of transvaginal ultrasonography. If conception had occurred, micronized progesterone supplementation was provided for an additional 4 weeks.

Weather

Weather parameters include temperature, relative humidity, atmosphere pressure and cumulative light hours. Weather information was acquired from the national weather bureau station of Kaohsiung, Taiwan.

Statistical Analysis

Statistical data was evaluated using SPSS 10.0 for windows (SPSS Inc., Chicago, IL, USA). Values are expressed as mean $\pm$ 2 standard deviation or percentages and proportions. Analysis of variance was performed for the demographic characteristics and IVF outcome parameters of the D3ET and D5ET. Pearson correlation analysis was used to identify correlations in the parameters tested. All tests were two tailed and a statistical value of $p < 0.05$ was considered to be significant.

RESULTS

We studied 647 couples who underwent their first ART treatment cycles, of which 325 (50.2%) were D3ET and 322 (49.8%) were D5ET. From this population, IVF and ICSI cycles account 201 (61.8%) and 124 (38.2%) for D3ET and 224 (69.6%) and 98 (30.4%) for D5ET respectively. The age (mean $\pm$ SD) of D3ET was 32.4 ± 3.9 and 32.0 ± 4.0 for D5ET ($p = 0.182$). The pathogenesis, IVF and ICSI cycles were not significant different between the two groups (Table I). The oocytes retrieved per cycle (mean $\pm$ SD) were 5.4 ± 3.7 for D3ET and 8.9 ± 4.3 for D5ET ($p < 0.001$). Day-3 embryo transfer has a higher number of embryos transferred than the D5ET (3.2 ± 1.4 vs. 2.9 ± 0.75 , $p < 0.001$). Day-5 embryo transfer group showed higher normal fertilization rate (NFR) (84.5% vs. 75.6%), higher top quality embryo rate (TQER) on day-3 (70.6% vs. 60.5%), higher implantation (29.7% vs. 16.9%) and clinical pregnancy rates (51.9% vs. 37.5%) than the day-3 embryo transfer group significantly.

Table II analyzes correlations of main outcome and weather parameters. Significant negative

Table I. Analysis of Age, Pathogenesis, Cycles Transferred, Normal Fertilization Rate, Top Quality Embryos, Pregnancy and Implantation Rates in Relation to Day-3 and Day-5 Embryo Transfers

Group	Day-3 ET	Day-5 ET	<i>p</i> Value
Age(years)	32.4 ± 3.9	32.0 ± 4.0	0.182
Pathogenesis			
Tubal factor	57 (17.5)	77 (23.9)	0.302
Male	91 (28.0)	85 (26.4)	0.874
Endometriosis	43 (13.2)	35 (10.9)	0.828
Ovulatory factor	23 (7.1)	21 (6.5)	0.985
Unexplained and others	52 (16.0)	71 (21.0)	0.467
Combined factors	59 (18.2)	33 (10.2)	0.153
Number of cycles transferred	325 (50.2)	322 (49.8)	—
IVF cycles (%)	201 (61.8)	224 (69.6)	0.296
ICSI cycles (%)	124 (38.2)	98 (30.4)	0.296
Oocytes retrieved/cycle	5.4 ± 3.7	8.9 ± 4.3	<0.001 ^a
Embryos transferred/cycle	3.2 ± 1.4	2.9 ± 0.75	<0.001 ^a
Normal fertilization rate (%)	1327/1756 (75.6)	2413/2857 (84.5)	<0.001 ^a
Day 3 TQER (%)	803/1327 (60.5)	1704/2413 (70.6)	<0.001 ^a
Day 5 TQER (%)	—	891/2413 (36.9)	—
Implantation rate (%)	181/1070 (16.9)	272/916 (29.7)	<0.001 ^a
Clinical pregnancy rate (%)	123/325 (37.5)	167/322 (51.9)	<0.001 ^a

Note. Values are mean ± SD or proportion and percentages in parentheses; Day-3 ET: patients with <3 good embryos on day-2 after oocyte retrieval; Day-5 ET: patients with ≥3 good embryos on day-2 after oocyte retrieval; TQER: top quality embryo rate.

^aStatistically significant.

correlations were observed both in the temperature with TQER of D3ET (−.289), day-3 and day-5 TQER of D5ET (−.575 and −.717, respectively), and in the implantation and pregnancy rates of D3ET (−.669 and −.818 respectively). Similar significant negative correlations appeared both in the relative humidity with TQER of D3ET (−.207), day-3 and day-5 TQER of D5ET (−.569 and −.738, respectively), and in the implantation and pregnancy rates of D3ET (−.389 and −.350, respectively), too. Additionally, relative humidity positively correlated with NFR of D5ET (.285) (Table II).

Atmosphere pressure changes inversely with temperature. It is therefore not surprising to see atmosphere pressure significantly correlated with TQER of D3ET (.181), day-3 and day-5 TQER of D5ET (.588 and .792, respectively), implantation and pregnancy rates of D3ET (.671 and .700, respectively). In addition to that, atmosphere pressure negatively correlated with NFR and pregnancy rate of D5ET (−.216 and −.112, respectively) (Table II).

The cumulative light hours negatively correlated with the implantation and pregnancy rates of D3ET (−.282 and −.282, respectively), while they positively

Table II. Correlation between Main Outcome Parameters and Weather

	Temperature	Relative humidity	Atmosphere pressure	Cumulative light hours
NFR of D3ET	.051	.005	−.057	−.093
NFR of D5ET	−.032	−.285 ^b	−.216 ^b	.125 ^a
Day 3 TQER of D3ET	−.289 ^b	−.207 ^b	.181 ^b	−.225 ^b
Day 3 TQER of D5ET	−.575 ^b	−.569 ^b	.588 ^b	−.218 ^b
Day 5 TQER of D5ET	−.717 ^b	−.738 ^b	.792 ^b	−.052
Implantation rate of D3ET	−.669 ^b	−.389 ^b	.671 ^b	−.282 ^b
Implantation rate of D5ET	−.081	−.032	−.053	.225 ^a
Pregnancy rate of D3ET	−.818 ^b	−.350 ^b	.700 ^b	−.282 ^b
Pregnancy rate of D5ET	−.05	.088	−.112 ^a	.224 ^b

Note. Values are Pearson correlation coefficient (*r*). NFR: normal fertilization rate; TQER: top-quality embryo rate; D3ET: day-3 embryo transfer; D5ET: day-5 embryo transfer.

^aCorrelation is significant at the 0.05 level.

^bCorrelation is significant at the 0.01 level.

Fig. 1. Change of weather parameters in months. hPa, hecto-pascal; Error bar, 2 standard deviation.

correlated with those of D5ET (.225 and .224, respectively). Besides, they negatively correlated with TQER of D3ET (−.225), and NFR and day-3 TQER of D5ET (−.125 and −.218, respectively) (Table II).

Figure 1 shows the change in weather parameters in months. Temperature was highest during the summer and was inversely related with atmosphere pressure (Fig. 1a and 1c). The relative humidity reaches its peak from June to August (Fig. 1b), and gradual declination of the cumulative light hours was observed from October to December (Fig. 1d). Main outcome parameters of D3ET and D5ET in relation to months were also shown in Fig. 2.

DISCUSSION

As expected and shown in Table I, the D5ET group had significantly more oocyte retrieved, better normal fertilization rate (NFR), more top quality embryos, higher implantation rate, and despite less embryos transferred, higher clinical pregnancy rate. This is in accordance with our previous report (12). The D5ET group was therefore the group with better ART outcome, as compared with the D3ET group.

The difference between the two groups might be due to the number of oocyte available and the embryo quality. It is well known that age has profound effects on the oocyte number and quality, and hence outcome of ART (15). The poorer prognosis of ART outcome may be inherent in the patients of day-3 group, in view of the similar age and pathogenesis distribution (Table I).

As shown in Fig. 1, there were quite long periods of high temperature and humidity, and low atmosphere pressure in southern Taiwan. Our laboratory suit was located deeply in the operating theater and the room temperature was controlled between 29–30°C by central air-conditioner and heater. The central air-conditioner was equipped with HEPA system to optimize the air quality and minimize the air blowing. The incubators and microscopes for handling and culturing oocytes and embryos were also stringently controlled by temperature and humidity. It is therefore unlikely that the laboratory condition could be affected by seasonal changes, and so it accounts for the observed differences in this study (Table II, Fig. 2).

Different geographical areas with different weathers may cause disparity in the results of IVF/ICSI

Fig. 2. Outcome of day-3 and day-5 embryo transfer in relation to months. Day-3 embryo transfers: patients with <3 good embryos on day-2 after oocyte retrieval. Day-5 embryo transfers: patients with ≥ 3 good embryos on day-2 after oocyte retrieval.

outcome. Better fertilization rates, embryo quality, and pregnancy rates during the cold months have been observed (16, 17). This is in accordance with the better TQER of both D3ET and D5ET groups during the cool seasons in the present study (Table II and Fig. 2). It appears that long and hot weather in tropical area may be detrimental to ART outcome generally. As expected accordingly, the D3ET group showed better implantation and pregnancy rates when the weather became cooler and darker. Surprisingly, in contrast to D3ET group, the implantation and pregnancy rates of D5ET group decreased during the cooler and darker months in this study (Table II and Fig. 2).

Melatonin, a hormone produced during the darkness, may be one of the factors which influence gonadal function (18). The involvement of melatonin in the steroid genesis of human ovaries has been documented (19,20). Webley and Luck (19) also reported direct stimulation of melatonin on hCG. Melatonin was thought to enhance hCG stimulation of progesterone production by human granulosa cells in culture (20). Yie *et al.* (21,22) reported higher concentrations of melatonin and progesterone during the dark season of the year and of oestradiol during the light season from pre-ovulatory follicular

fluids. Rojansky *et al.* (4), first correlates the absolute number of light hours with fertilization rate and the result was highest in spring and lowest in autumn. The absolute number of light hours was not included as a weather parameter in our study. But since the change in light hours of the tropics is not as evident as the northern countries, its effect upon the concentration of melatonin in the follicular fluids needs further investigation.

Better sperm quality in the cooler seasons were observed by many authors (23–25). Deterioration of sperm quality may cause lower conception rate especially in hot summer of sub-equatorial areas (3). Mild poor sperm quality could be treated with IVF. For those with severe poor sperm quality, normal fertilization could be easily achieved by ICSI. The impacts of poor sperm quality on ART outcome thus may be largely eliminated in the treatment of ART (26,27). Therefore, we did not evaluate further the situation of the sperm quality in this study.

The implantation and clinical pregnancy rates of D3ET decreased when the temperature increases (Fig. 1a, 2c and 2d). Embryo quality might account for the difference in implantation and pregnancy rates as the TQER changed in the same direction (Table II). Whether endometrial receptivity also

played a role needs further verification. It has been reported that endometrial receptivity may be reduced during the long, dark winters in some northern countries (28,29). But the effects of long, hot temperature, and low atmosphere pressure on endometrial receptivity have never been thoroughly investigated.

The implantation and pregnancy rates of the D5ET changed in the opposite direction of D3ET. This is hard to explain since both groups showed improved embryo quality during cool, dark months (Table II, Fig. 2). We have routinely offered blastocyst transfer to patients with ≥ 3 good four-cell embryos on day-2 after oocyte retrieval. Patients with < 3 good four-cell embryos on day-2 received day-3 embryo transfer at the 8-cell stage. The D5ET group in this study was therefore having better ART outcome than the D3ET group (Table I). The inherent difference in the ovarian response between these two groups may account for different responses to weather change. Moreover, embryos capable of reaching blastocyst stage for transfer are in greater chance of success. It has been recognized that there were better synchronization between embryo and endometrium, and better embryo quality in blastocyst transfer (30). Whether or not prolonged in vitro culture to day-5 modified the influences upon embryo quality or endometrial receptivity from weather environment requires further investigation. Thirdly, the patient number was relatively small when divided into each month. More cases are needed in further studies to confirm or dispute our findings.

In conclusion, the present study, to our knowledge, is the first report demonstrating impacts of tropic weather temperature, humidity, atmosphere pressure, and cumulative light hours upon patients with different ART outcome. It appears that long hot weather in the tropical area may be detrimental to ART outcome generally. Better ovarian response or blastocyst culture and transfer may modify weather impacts upon ART outcome. If weather does have different impacts upon different patient groups, then it should be taken into consideration at the time of ART treatment.

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