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Lack of seasonal variations in fertilization, pregnancy and implantation rates in women undergoing IVF

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BACKGROUND: Several studies have investigated seasonal variations during IVF. Their results are contradictory, especially concerning fertilization and pregnancy rates. The aim of the present study was to re-evaluate these parameters using a large number of IVF cycles. METHODS: A total of 7368 IVF cycles conducted in Switzerland between 1995 and 2003 were retrospectively analysed. To avoid a bias in the evaluation of the fertilization rate, only IVF cycles without ICSI were considered for analysis. Cycles were assigned to seasons according to the date of the beginning of stimulation. RESULTS: There were no statistically significant differences between the seasons concerning the fertilization, the pregnancy and the implantation rates. However, statistically significant variables deciding on the outcome of an IVF cycle are age, centre, aetiology of infertility and day of transfer. CONCLUSIONS: There were no statistically significant seasonal differences in central Europe (Switzerland) that influenced the outcome of IVF treatment. The only statistically significant variables of IVF outcome were age, centre, aetiology of infertility and day of transfer. A change to routine fertility treatment concerning the different seasons should therefore not be taken into account.

Key words: fertilization rates/implantation rates/IVF/pregnancy rates/seasonality

Introduction

Every physician and biologist working in the field of reproductive medicine keeps wondering about variable pregnancy rates, because success rates are fluctuating without visible reason from excellent to very poor. This is often not explained by the group a patient belongs to (e.g. advanced age of the woman, poor responders, primary infertility of the female, multiple infertility factors) nor by cultural conditions, technical failures or other comprehensible reasons. Thus, the question arises whether the different seasons of the year can have good or bad influences on the characteristics of the oocytes and on the fertilization and pregnancy rates during assisted reproductive technologies in humans. If this were the case, it would be possible to avoid IVF treatment cycles in the 'bad months' in order to enhance pregnancy rates.

All over the world, seasonal changes in the reproductive life of animals are well known. The question arises whether human reproduction is also affected by such seasonal differences, although sexual activity of human beings is not bound to seasons. Several epidemiological studies point to a variation in natural conception and birth rates not only in animals but also in humans (Odegard *et al.*, 1977; Mathers and Harris, 1983; Roenneberg *et al.*, 1990a,b; Lam *et al.*, 1991), being reduced during the spring in warm climates in non-equatorial regions (Huntington, 1938; Lamar *et al.*, 1943; Rosenberg, 1966; Becker, 1981). The reasons for this phenomenon are not very well understood; possible explanations might be the variations in semen quality, showing a significantly lower sperm count in the summer compared to the winter (Tjoa *et al.*, 1982; Levine *et al.*, 1988; Reinberg *et al.*, 1988; Politoff *et al.*, 1989; Saint Pol *et al.*, 1989; Levine *et al.*, 1990; Levine, 1991), variations in the ovulation rate (Timonen *et al.*, 1964; Kivela *et al.*, 1988; Rameshkumar *et al.*, 1992) and variations in endometrial function (Timonen *et al.*, 1964; Kottler *et al.*, 1989) throughout the seasons: these variations might be linked to the light–dark effect of the female reproductive axis (Rojansky *et al.*, 1992). On the other hand, inconsistent frequencies of intercourse (Odegard *et al.*, 1977; Ehrenkranz *et al.*, 1983; Abas and Murphy, 1987; Jacobsen *et al.*, 1987), possibly increasing during holidays (Rosenberg, 1966; Wrigley and Schofield, 1981; Cesario, 2002), might also be an explanation. Others, however, did not confirm the occurrence of varying frequencies of intercourse as a function of season (Udry *et al.*, 1967; Levine *et al.*, 1990).

Studies on reproductive outcome of assisted technologies in primates have shown significant variations throughout the seasons, the oocyte maturation being much poorer in winter months (Smith *et al.*, 1978; Chan *et al.*, 1982). The most intriguing finding in these above-mentioned studies is the persisting seasonal effect on the maturation of retrieved oocytes during

IVF, despite the administration of exogenous gonadotrophins and constant environmental conditions.

Possible seasonal variations during assisted reproductive treatment of humans have been suggested by several studies (Wood *et al.*, 1985; Stolwijk *et al.*, 1994; Chamoun *et al.*, 1995; Ossenbühn, 1998; Rojanski *et al.*, 2000; Weigert *et al.*, 2001), although variations of the ovulation rate due to varying endogenous gonadotrophin secretion are suppressed by the hormonal therapy given during IVF treatment. Furthermore, lower sperm counts in non-treated ejaculates (observed during the summer months) are compensated in IVF treatment cycles by utilizing a concentrated and constant amount of motile sperm to inseminate the oocytes. Other studies could not confirm the presence of seasonal variations (Casper *et al.*, 1988; Daya *et al.*,1993; Fleming *et al.*, 1994).

Considering these controversial data, we retrospectively evaluated the outcome of 7368 IVF cycles, conducted over a 9 year period throughout Switzerland and encompassing all four seasons of the year.

Materials and methods

Patients

A total of 7368 IVF cycles, conducted in all 18 IVF clinics of Switzerland between 1995 and 2003, were retrospectively analysed (4688 patients, 62.3% with one cycle, 25.5% with two, 7.1% with three, 5.1% with more than three cycles). For this analysis, the national IVF data register (FIVNAT) has been used.

To avoid a bias in the evaluation of the fertilization rate, only IVF cycles were considered for analysis. No cycles with ICSI were included. The indications for the IVF treatment were tubal factors in 34.6%, male factor in 11.7%, idiopathic infertility in 9.5%, endometriosis in 5.1% and multiple causes in 29.7%, while other causes accounted for 9.4%. Patients had in 60.7% of the cycles a primary infertility and in 39.3% a secondary infertility.

The decision for IVF treatment had been made before the start of the study and all patients have been treated routinely. Because all data have been rendered anonymous when entered in the computerized data base, approval of the ethical committee was not necessary.

Methods

The seasons were defined before analysing the data, according to the calendar definitions of the seasons for Europe, each season lasting 3 months: Spring: March 21–June 20; Summer: June 21–September 20; Autumn: September 21–December 20; Winter: December 21–March 20.

Because the season may not only have an impact on embryo development and implantation, but also influence the oocyte during its development at the time of IVF treatment, the attribution of the patients to the corresponding seasons was made according to the date of the beginning of stimulation.

Complete infertility investigation of both partners (hormonal evaluation, gynaecological ultrasound, hysterosalpingography or laparoscopy to evaluate tubal patency and semen analysis) preceded the IVF treatment.

In all, 68.2% of patients were stimulated with the long protocol, 32.8% with the short protocol. Down-regulation was achieved with triptorelin (daily injection or depot preparation; Decapeptyl®, Ferring pharmaceuticals, Wallisellen, Switzerland), goserelin depot preparation (Zoladex®, Astra Zeneca, Zug, Switzerland) or nafarelin nasal spray (Synrelina®, Pharmacia, Dübendorf, Switzerland). Gonadotrophins used for stimulation were in 24.3% recombinant FSH (Gonal-F®, Serono Pharma, Geneva, Switzerland; or Puregon®, Organon Pharmaceuticals, Pfäffikon, Switzerland), in 34.2% highly purified FSH (Metrodin HP®, Serono; Fostimon®, IBSA, Pambio-Noranco, Switzerland) and 40.4% HMG, (Pergonal®, Serono; Humegon®, Organon; Menogon®, Ferring pharmaceuticals; Merional®, IBSA). The different stimulation protocols and gonadotrophins were statistically equally distributed among the different groups of patients divided according to the seasons, thus excluding any bias.

Thirty-five to 36 h after hCG administration, oocytes were retrieved by needle aspiration, with transvaginal ultrasound guidance and under routine intravenous sedation or general anaesthesia.

Cycles with micromanipulation (e.g. ICSI, subzonal injection of sperm etc.), with gamete or zygote intra-Fallopian transfer as well as cryocycles were excluded. The laboratory work (preparation of oocytes and the ejaculate, fertilization, incubation and embryo transfer) was performed by the use of previously described standard techniques (Lewin *et al.*, 1986). Fertilization was assessed 17 h (range 15–20) after IVF. Only normal pronucleids [normally fertilized oocytes with two pronuclei (PN) and two polar bodies] were considered for embryo transfer and freezing was done if more than two or three pronucleids were available. Those with the highest PN score and with the best morphological grade were selected for transfer in each treatment cycle. They were cultured for another 20–30 h at 37°C in fresh CO_2-equilibrated IVF medium. All remaining pronucleids were cryopreserved in the 4-cell embryo or PN stage, for later transfers. Since the beginning of the year 2001, no embryos, but only pronucleids, are allowed to be frozen according to Swiss law.

The pregnancy rate after IVF is defined by the proportion of patients with a positive HCG value, ≥14 days after embryo transfer. The fertilization rate is defined by the proportion of only mature oocytes with one polar body resulting in pronucleids. The implantation rate is defined by the proportion of the number of embryos transferred resulting in gestational sacs ultrasonographically diagnosed 2 weeks after the positive pregnancy test, including ectopic gestations. Mean embryo quality could not be calculated, because the different IVF centres used different classifications for the evaluation of the embryo quality, making statistical analysis impossible.

Statistical methods

Categorical variables were compared for homogeneity within seasons and months using χ^2 for goodness of fit. Continuous variables were compared using one-way parametric analysis of variance and multivariate regression. IVF results of the cycles in the different seasons and months were assessed first using χ^2 goodness of fit and subsequently using a multivariate logistic model adjusting for centre, age, indication, infertility type and year of stimulation. In a subsequent analysis considering only cycles with embryo transfer, two additional covariates—the number of transferred embryos and the day of transfer—were considered. The significance level was set to $\alpha = 0.05$ two-tailed and all calculations were performed using SAS vs 8.02.

All statistical analyses were repeated considering the first treatment cycle only.

Results

A total of 7368 IVF cycles (first treatment cycle in 62.2%, second cycle in 25.6%, ≥ third cycle in 12.2%) were analysed. To exclude any potential bias due to repeated observations, a subsequent statistical sub-analysis was carried out for the 4688 IVF first cycles. The results from the sub-analysis performed with the 4688 first cycles did not differ from those obtained by

the analysis of all the cycles (data not shown). Therefore, all IVF cycles could be evaluated.

Table I shows the distribution of the clinical parameters of the patients (age, primary/secondary infertility, aetiology of infertility and number of IVF cycles) in the different seasons. There were no significant differences for the type of infertility (primary/secondary) within the seasons, but the aetiology of infertility ($P < 0.01$) and the number of IVF cycles conducted within the different seasons varied significantly. The number of IVF cycles performed was lowest in summer and autumn and highest in winter and spring ($P < 0.0001$).

Table II presents the results of the number of IVF cycles, the number of oocytes retrieved, the number of pronucleids obtained, the number of embryos transferred, the pregnancy rates and the implantation rates for the IVF cycles done in the different seasons. Pregnancy rates were not found to be different between the seasons.

Table III presents the results of the pregnancy and implantation rates for IVF cycles per month during the whole observation period. There were no significant differences in pregnancy and implantation rates, but there was in the number of embryos transferred. Also after correction for the number of embryos, age, infertility type (primary/secondary), aetiology of infertility and day of transfer, there were no significant differences in adjusted pregnancy and implantation rates (data not shown).

Table IV shows the distribution of the number of IVF cycles, the age of the patients, the type infertility (primary/secondary), the aetiology of infertility, the number of oocytes retrieved, the number of pronucleids obtained and the number of embryos transferred in the different years of the observation period. For all these parameters—with the exception of the type of infertility—significant differences have been seen between years of stimulation.

Table V presents the pregnancy results of all initiated IVF cycles adjusted for season, age of the patient, infertility type, treatment indications, year of treatment and centre. The odds of becoming pregnant do not differ between the season (OR 0.98; CI: 0.83–1.17 for summer, OR 1.02; CI: 0.85–1.21 for autumn and OR 1.11; CI: 0.94–1.31 for winter, taking spring as reference), whereas age, indication for treatment, year of stimulation and centre are significant factors: the odds of a patient aged <30 years are 4.4-fold those of a patient aged ≥40 years (OR CI: 3.6–5.7), for patients aged 30–34 years they are 3.4-fold (OR CI: 2.7–4.3) and for patients aged 35 years 2.8-fold (OR CI: 2.3–3.6). The odds for the indication for treatment are unfavourable for a male factor (OR 0.74; CI 0.60–0.91).

Table I. Distribution of the age of the patients, infertility type (primary/secondary), aetiology of infertility (treatment indications) and number of IVF cycles in the different seasons.

Clinical parameter	Spring	Summer	Autumn	Winter	*P*
Age of patients (mean ± SD)	35.5 ± 4.4	35.5 ± 4.2	35.0 ± 4.5	35.6 ± 4.4	<0.0001
Infertility type					
Primary	59.3	61.2	62.2	60.3	NS
Secondary	40.7	38.8	37.8	39.7	NS
Treatment indications					
Multiple causes	30.5	29.8	28.1	30.3	NS
Tubal	36.1	34.1	34.6	33.6	NS
Male	10.9	10.3	12.4	12.8	NS
Endometriosis	5.6	5.2	4.6	5.1	NS
Idiopathic	7.9	10.6	10.8	8.9	0.01
Others (not specified)	9.0	10.1	9.4	9.3	NS
Initiated IVF cycles [*n* (%)]	1895 (25.7)	1667 (22.6)	1764 (23.9)	2042 (27.7)	<0.0001

Values are percentages unless otherwise specified.
P-Values were obtained by one-way ANOVA for continuous variables and goodness of fit χ^2 test for categorical variables.
NS = not significant.

Table II. Number of initiated cycles, number of oocytes retrieved, number of pronucleids (normally fertilized oocytes with two pronuclei and two polar bodies) obtained, number of embryos transferred, pregnancy rates and implantation rates for IVF cycles in the different seasons

Clinical parameter	Spring	Summer	Autumn	Winter	*P*
No. of initiated IVF cycles	1895	1667	1764	2042	<0.0001
Cycles with oocyte retrieval (% of initiated cycles)	1730 (91.3)	1539 (92.3)	1622 (92.0)	1864 (91.3)	<0.0001
Cycles with transfer (% of initiated oocyte retrieval cycles)	1544 (81.5)	1343 (80.6)	1435 (81.3)	1693 (82.9)	<0.0001
Oocytes retrieved (mean ± SD)	8.8 ± 6.1	8.5 ± 5.9	9.2 ± 6.6	8.5 ± 5.8	NS
Pronucleids obtained (mean ± SD)	5.2 ± 4.3	4.9 ± 4.2	5.1 ± 4.4	5.0 ± 4.1	NS
Embryos transferred (mean ± SD)	1.9 ± 1.1	1.8 ± 1.1	1.8 ± 1.0	1.9 ± 1.0	NS
Clinical pregnancies	361	322	353	411	
Pregnancy rate by initiated cycle	19.1	19.3	20.0	20.1	NS
Pregnancy rate by oocyte retrieval	20.9	20.9	21.8	22.0	NS
Pregnancy rate by transfer cycle	23.4	24.0	24.6	24.3	NS
Implantation rate	13.1	13.4	13.2	14.3	NS

Values given are mean and range; *P* values for continuous variables were obtained by Student's *t*-test and for categorical variables with χ^2 for goodness of fit.
NS = not significant.

Table III. Pregnancy and implantation rates for all IVF cycles by month over the entire observation period

Clinical parameter	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	*P*
Initiated cycles	715	737	714	637	766	414	371	655	737	731	669	222	<0.0001
Cycles with oocyte retrieval (% of initiated cycles)	91.9	91.6	91.6	91.2	90.3	92.5	91.9	93.0	90.0	92.9	93.7	87.4	<0.0001
Cycles with transfer (% of initiated oocyte retrieval cycles)	82.4	82.8	85.4	81.5	79.0	82.9	79.2	81.4	77.9	83.3	82.7	79.3	<0.0001
Oocytes retrieved (mean ± SD)	8.3 ± 5.4	8.8 ± 6.0	8.4 ± 5.8	8.5 ± 5.7	8.9 ± 6.1	8.7 ± 6.3	9.1 ± 6.6	8.5 ± 5.6	8.3 ± 6.1	9.3 ± 6.8	9.5 ± 6.6	9.3 ± 6.8	NS
Pronucleids obtained (mean ± SD)	4.8 ± 3.8	5.2 ± 4.2	5.0 ± 4.2	5.1 ± 4.3	5.3 ± 4.4	5.0 ± 4.2	5.1 ± 4.5	4.7 ± 4.0	4.8 ± 4.2	5.2 ± 4.4	5.3 ± 4.6	5.5 ± 4.4	NS
Embryos transferred (mean ± SD)	1.9 ± 1.0	1.9 ± 1.1	1.9 ± 1.0	1.8 ± 1.1	1.8 ± 1.1	1.9 ± 1.1	1.8 ± 1.1	1.8 ± 1.1	1.7 ± 1.1	1.8 ± 1.0	1.9 ± 1.0	1.7 ± 1.0	0.01
Clinical pregnancies	148	157	145	109	143	82	68	125	147	140	128	55	
Pregnancy rate by initiated cycle (%)	20.7	21.3	20.3	17.1	18.7	19.8	18.3	19.1	19.9	19.2	19.1	24.8	NS
Pregnancy rate by oocyte retrieval (%)	22.5	23.3	22.2	18.8	20.7	21.4	19.9	20.5	22.2	20.6	20.4	28.4	NS
Pregnancy rate by transfer cycle (%)	25.1	25.7	23.8	21.0	23.6	23.9	23.1	23.5	25.6	23.0	23.1	31.3	NS
Implantation rate (%)	14.7	15.1	13.5	12.0	12.8	14.1	12.5	13.6	13.9	12.9	11.8	17.8	NS

NS = not significant.

Table IV. Distribution of the clinical parameters and number of IVF cycles in the different years of the observation period

Clinical parameter	1995	1996	1997	1998	1999	2000	2001	2002	2003	*P*
No. of IVF cycles	662	737	952	960	852	829	756	864	756	<0.0001
Age of patients (years) (mean ± SD)	34.8 ± 4.4	34.7 ± 4.4	34.7 ± 4.3	35.2 ± 4.3	35.5 ± 4.3	35.9 ± 4.4	35.9 ± 4.4	36.0 ± 4.4	35.9 ± 4.5	<0.0001
Type of infertility										
Primary	62.8	60.8	62.3	62.2	58.0	61.2	58.6	60.1	60.3	NS
Secondary	37.2	39.2	37.7	37.8	42.0	38.8	41.4	39.9	39.7	NS
Treatment indications										
Multiple causes	28.7	23.6	25.2	22.4	24.2	29.1	44.3	38.0	34.3	<0.0001
Tubal	28.9	35.1	33.5	38.1	39.1	36.8	31.6	33.3	33.2	0.015
Male	17.4	14.0	12.8	11.3	11.2	11.0	7.8	8.1	12.7	<0.0001
Idiopathic	5.0	4.7	4.8	5.2	5.8	5.1	2.4	6.4	6.7	0.003
Endometriosis	7.3	9.1	9.9	9.1	12.0	11.2	9.0	7.3	10.1	0.017
Others (not specified)	12.8	13.4	13.8	14.0	7.9	6.9	4.9	6.9	3.0	<0.0001
Oocytes retrieved (mean ± SD)	8.4 ± 6.0	8.4 ± 5.9	8.0 ± 5.4	8.4 ± 5.9	8.7 ± 6.0	8.9 ± 5.9	9.8 ± 7.1	9.1 ± 6.4	9.3 ± 6.1	<0.0001
Pronucleids obtained (mean ± SD)	5.1 ± 4.4	4.9 ± 4.2	4.5 ± 3.9	4.7 ± 4.0	4.8 ± 4.2	5.1 ± 4.3	5.7 ± 4.7	5.5 ± 4.4	5.4 ± 4.2	<0.0001
Embryos transferred (mean ± SD)	2.1 ± 1.2	2.0 ± 1.1	1.9 ± 1.1	1.8 ± 1.1	1.8 ± 1.1	1.9 ± 1.0	1.8 ± 1.0	1.7 ± 0.9	1.7 ± 0.9	<0.0001
Pregnancy rate by initiated cycle	19.8	18.9	15.0	17.1	19.1	20.5	19.4	24.7	23.4	<0.0001
Pregnancy rate by oocyte retrieval	21.1	20.5	16.1	18.6	20.7	22.5	22.0	27.2	25.7	<0.0001
Pregnancy rate by transfer cycle	24.0	23.1	18.3	21.3	24.0	24.7	23.9	30.0	28.5	<0.0001
Implantation rate	12.8	12.0	9.6	11.7	13.7	13.8	14.1	17.9	17.7	<0.0001

NS = not significant.

Table V. Pregnancy status for IVF-initiated cycles by season: multivariate odds ratios

Factors	Point estimate	95% confidence interval	
		Lower	Upper
Season (reference class: spring), $P = 0.447$ (NS)			
Summer	0.981 (NS)	0.825	1.165
Autumn	1.015 (NS)	0.853	1.208
Winter	1.110 (NS)	0.939	1.313
Age (years) (reference class: ≥40 years), $P < 0.0001$			
<30	4.375[a]	3.359	5.698
30–34	3.429[a]	2.713	4.333
35–39	2.840[a]	2.259	3.570
Indication (reference class: tubal), $P = 0.0066$			
Endometriosis	0.959 (NS)	0.726	1.267
Idiopathic	1.207 (NS)	0.976	1.492
Male	0.737[a]	0.596	0.911
Others (not specified)	0.957 (NS)	0.829	1.104
Infertility type (reference class: primary), $P = 0.0734$ (NS)			
Secondary	1.122 (NS)	0.989	1.272
Year (reference class: 1995), $P = 0.0015$			
1996	0.888 (NS)	0.678	1.165
1997	0.628[a]	0.4801	0.823
1998	0.722[a]	0.554	0.941
1999	0.781 (NS)	0.597	1.023
2000	0.819 (NS)	0.625	1.072
2001	0.782 (NS)	0.592	1.032
2002	1.053 (NS)	0.806	1.376
2003	0.954 (NS)	0.738	1.285
Centre, [b]$P < 0.0001$			

[a]Significant favourable/unfavourable factor, $P < 0.05$.
[b]Detailed odds ratios by centre not shown.
NS = not significant.

The year of stimulation is also a significant factor: being stimulated in 1997 (OR 0.6; CI 0.5–0.8) or 1998 (OR 0.7; CI 0.6–0.9) gives significantly worse odds for a pregnancy, as compared to 1995.

Table VI presents the pregnancy results limited to IVF cycles with embryo transfer adjusted for season, age of the patient, infertility type, treatment indication, number of transferred embryos, day of transfer, year of treatment and centre. The odds of becoming pregnant do not differ between the seasons (OR 0.94; CI: 0.79–1.13 for summer, OR 1.00; CI: 0.83–1.20 for autumn and OR 1.09; CI: 0.91–1.30 for winter, taking spring as reference). In contrast, age, indication for treatment, number of transferred embryos, day of transfer, year of stimulation and centre are significant factors deciding on the outcome: the odds of a patient aged <30 years are 4.0-fold those of a patient aged ≥40 years (CI 3.0–5.3), for patients aged 30–34

Table VI. Pregnancy status for IVF cycles with embryo transfer by season: multivariate odds ratios

Factors	Point estimate	95% confidence interval	
		Lower	Upper
Season (reference class: spring), $P = 0.4297$ (NS)			
Summer	0.943 (NS)	0.788	1.129
Autumn	0.999 (NS)	0.833	1.198
Winter	1.086 (NS)	0.912	1.294
Age (years) (reference class: ≥40 years), $P < 0.0001$			
<30	3.979[a]	3.014	5.252
30–34	2.898[a]	2.272	3.696
35–39	2.496[a]	1.969	3.163
Indication (reference class: tubal), $P = 0.0453$			
Endometriosis	1.051 (NS)	0.787	1.404
Idiopathic	1.290[a]	1.033	1.611
Male	0.852 (NS)	0.682	1.064
Others (not specified)	1.081 (NS)	0.932	1.255
Infertility type (reference class: primary), $P = 0.4863$ (NS)			
Secondary	1.048 (NS)	0.919	1.193
No. of embryos transferred (reference class: 2), $P < 0.0001$			
1 embryo	0.389[a]	0.301	0.503
≥3 embryos	1.175[a]	1.008	1.371
Day of transfer (reference class: day 2), $P = 0.0119$			
Day 3	1.211 (NS)	0.997	1.470
Day 4 or later	1.687[a]	1.161	2.451
Year (reference class: 1995), $P = 0.0107$			
1996	0.919 (NS)	0.693	1.217
1997	0.697[a]	0.526	0.924
1998	0.793 (NS)	0.598	1.052
1999	0.838 (NS)	0.627	1.120
2000	0.795 (NS)	0.593	1.065
2001	0.776 (NS)	0.573	1.051
2002	1.124 (NS)	0.835	1.513
2003	1.019 (NS)	0.749	1.385
Centre, [b]$P < 0.0001$			

[a]Significant favourable/unfavourable factor, $P < 0.05$.
[b]Detailed odds/ratios by centre not shown.
NS = not significant.

years they are 2.9-fold (CI 2.3–3.7) and for patients aged 35 years 2.5-fold (OR CI 2.0–3.2). The odds for the indication for treatment are more favourable to idiopathic (OR 1.3; CI 1.0–1.6) when taking as reference the female indication.

The number of transferred embryos is also a significantly predictive factor: when taking as reference two transferred embryos the odds are significantly unfavourable for one embryo (OR 0.4; CI 0.3–0.5) and favourable for three or more embryos (OR 1.2; CI 1.0–1.4). The day of transfer is a significantly favourable factor when the transfer is done on day 4 or later, as compared to day 2 (OR 1.7; CI 1.2–2.5), while transfers performed on day 3 do not show a significant advantage compared to day 2 (OR 1.21; CI: 1.00–1.47). The year of stimulation is also a significant factor: being stimulated in 2002 (OR 1.7; CI 1.3–2.2) or 2003 (OR 1.5; CI 1.2–2.0) gives significantly higher odds for pregnancy, as compared to 1995.

Discussion

Studying carefully the literature on seasonal variations in the outcome of IVF cycles, the extent of discrepancies between these studies is remarkable. One point is the difference in the stimulation protocols used. In some studies, cycles with down-regulation were included, in others not. In addition, there was no agreement between the studies about the criterion for the inclusion of a patient to the corresponding season: in some studies it was the day of the beginning of stimulation, in others it was the day of oocyte retrieval, resulting in a difference up to 17 days. Also, the published studies have been conducted in different climates. Because of the widely diverse environments with different temperatures and light changes, the different studies are not comparable. Regarding the heterogeneous study results, it is also remarkable that the seasonal effects observed are very complex. Rojanski *et al.* (2000) for example showed that even though the fertilization and the embryo quality rates were best in spring, the pregnancy rates were worst in spring. On the other hand, he found the worst fertilization and worst embryo quality rates in autumn, but the best pregnancy rates at that time (Rojanski *et al.*, 2000).

The results between the different studies were highly inconsistent, some showing the best pregnancy rates in the months of November, December, January and February (Stolwijk *et al.*, 1994), others showing the worst pregnancy rates in the months of January, February and March in IVF cycles with spontaneous LH surge (Casper *et al.*, 1988), whereas no differences were seen in IVF cycles with HCG administration (Casper *et al.*, 1988).

Our results showed no significant seasonal differences in the fertilization rate nor in the pregnancy or implantation rates. Our findings are in agreement with other studies also showing

no differences in the results of assisted reproductive techniques done in the different seasons of the year (Casper *et al.*, 1988; Daya *et al.*, 1993; Fleming *et al.*, 1994). One explanation why no differences in IVF success rates were found is that the lower sperm count observed during the summer months in non-treated ejaculates is compensated in IVF by utilizing a concentrated and constant amount of motile sperm to inseminate the oocytes. Another explanation is the elimination of a seasonal influence on oocyte growth and ovulation by the suppression of the hypothalamic–pituitary axis through down-regulation by GnRH analogues and the exogenous administration of gonadotrophins for ovulation induction during the IVF cycle.

Our results also show no significant seasonal differences in the implantation rate, pointing to an absence of a seasonal change in endometrial receptivity.

Comparing our analysis of the different months, the best pregnancy and implantation rates were found in December, despite a significantly lower number of embryos transferred. However, these differences did not reach statistical significance. In a study conducted in Austria (Weigert *et al.*, 2001), having roughly the same climate as Switzerland and having also included a large number of patients in a similar time-frame (8184 IVF cycles from 1992 to 1999), the best pregnancy rates were also found in December, and reaching statistical significance. Our results correspond only partly to the analysis of Stolwijk *et al.* (1994) from The Netherlands (quite a similar climate, too) who reported a better fertilization rate, embryo quality and pregnancy rate in the period from November to February. These European findings are difficult to explain. One hypothesis of seasonal fluctuations in fertilization and pregnancy rates is the correlation with the annual changes in sunlight exposition; hypothalamic–pituitary output, neurotransmitters and melatonin are suspected to be causally related. The mechanism of the suspected seasonal variation in human fertility has been attributed to a direct melatonin or neurotransmitter effect on the end-organ (Kauppila *et al*., 1987; Yie *et al*., 1995; Malpaux *et al*., 1999), but the role of melatonin in reproduction has been not well defined until now. However, influences of the light/dark cycle on the female reproductive process have been demonstrated (Timonen *et al.*, 1964; Kivela *et al.*, 1988; Kottler *et al.*, 1989; Rojansky *et al.*, 1992), with a reduced ovulation rate and endometrial receptivity in winter months in spontaneous cycles. These findings are in frank contradiction to the cited studies on IVF cycles, where the best pregnancy rates were found in the winter. However, spontaneous cycles cannot be compared with IVF cycles, because of the suppression of the hypothalamic–pituitary axis through down-regulation by GnRH analogues and the exogenous administration of gonadotrophins for ovulation induction during in assisted reproduction treatment.

Our finding of the significantly different aetiologies between the seasons is difficult to explain and might be a coincidence. In contrast, the decrease of the male factor as an indication for IVF treatment from 1995 to 2003 is well explained by the increased use of ICSI for this aetiology since the mid-1990s.

Comparing the success rates in the different years of the observation period, a big increase in the quality of IVF treatment can be observed. The pregnancy rate increased significantly even though the number of transferred embryos significantly decreased and the mean patient age significantly increased. There exist also statistically significant differences in the success rates between the different centres, but no single IVF centre showed a seasonal variation or other statistically significant variations during the year in its results.

Our results showed that the statistically significant variables influencing the outcome of an IVF cycle are age, aetiology of infertility, day of transfer and centre. This concurs with the assisted reproductive technologies data report of 2002 (http://www.cdc.gov/reproductivehealth/ART02/PDF/ART2002.pdf), conducted by the Centers for Disease Control.

In conclusion, by the analysis of a large number of IVF cycles, we confirmed that statistically significant variables for IVF outcome are age, aetiology of infertility, the day of transfer and centre. However, the suspected seasonal variability of the outcome of IVF cycles has not been confirmed: there is no statistically significant variability in fertilization, implantation or pregnancy rates between the seasons in IVF. A change of routine fertility treatments concerning the different seasons should therefore not be taken into consideration.

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20 IVF centres (Baden; Basel (3); Bellinzona; Bern (2); Chene-Bougeries; Geneve; Kreuzlingen; Lausanne (2); Locarno; Lucern; Schaffhausen; St Gallen; Winterthur; Zurich (3)) of the FIVNAT-CH group participated in the study.

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