Lower Pregnancy Rates After IVF in Women Working as Occupational Cleaners A Preliminary Longitudinal Study

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A Preliminary Longitudinal Study

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OBJECTIVE: To assess clinical, biological, and environmental factors influencing pregnancy rates (PRs) after in vitro fertilization (IVF), and to study the influence of occupational, lifestyle, and domestic exposure on PR.

STUDY DESIGN: A longitudinal cohort study was performed in women who started an IVF cycle with a self-administered questionnaire concerning their environmental and occupational exposure. Medical data were obtained from medical files.

RESULTS: Among 534 cycles, we showed a variety of factors that had an impact on PR: age, infertility duration, number of mature oocytes and embryos. After multivariate analysis, women with "elementary" occupations had a significantly lower PR (OR 5.6; 95% CI 1.3–23.7). Among them, 82% were cleaners.

CONCLUSION: This preliminary result leads us to focus on a socio-professional category that is already recognized in the literature as at risk for congenital abnormalities during pregnancy. Further cohort studies are needed to study the influence of socio-professional category on PR.

… pregnancy rates were significantly reduced in women working an "elementary" occupation.

Keywords: assisted reproductive technologies, infertility, in vitro fertilization, IVF, pregnancy rate, occupational exposure.

As with natural fertility, many factors may affect pregnancy rates (PRs) in in vitro fertilization (IVF). The main prognostic factors in women are age, body mass index, and tobacco use.[1-3] Chances of pregnancy after IVF are better in women who are <34 years old.[4] However, several clinical and biological factors related to the etiology of the infertility[5] and the IVF procedure influence PR, such as the intracytoplasmic sperm injection (ICSI) operator, the number and the quality of embryos trans-

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ferred, and the experience of the embryo transfer provider.[6-8]

The impact of environmental factors is difficult to study because of the variety and large number of environmental exposures and the difficulty in proving the causal link because of a lack of specific exposure biomarkers. Tobacco smoke is widely recognized to reduce PRs in IVF,[3] but the impact of environmental and occupational exposure on IVF PR has not been well-studied.[9,10] Concerning solvents, in animal models they are suspected to impair reproductive functions: for example, in rats, 2-bromopropane treatment increased estrous cycles, decreased the number of oocytes in spontaneous ovulation, the number of pups born, and uterine weights.[11,12] In humans, exposure to toxic environmental agents can interfere with all developmental stages of reproductive functions in adult females,[13,14] and Koh et al reported that a toxic occupational exposure to cleaning solvent could be responsible for premature ovarian failure.[15] To our knowledge, there is no study assessing the relationship between occupational exposure and IVF outcome.

The objectives of our study were first to assess clinical, biological, and environmental factors that could influence IVF PR, and secondly to study if occupational exposure had an impact on IVF PR.

Materials and Methods

We conducted a longitudinal cohort study in our assisted reproductive technologies center in a university teaching hospital in Marseille, France, between January 2013 and February 2014. Ethical approval for the study was obtained from the French Obstetric and Gynecologic Research Ethic Committee (CEROG 2011-GYN-05-02-R1).

Participants were given a self-administered questionnaire concerning clinical, biological, and environmental factors known to impair ovarian reserve and female fertility (questionnaire available upon request).[13,14,16,17] Inclusion criteria were women aged 18–43 years who spoke and read French fluently and who started an IVF cycle regardless of the attempt rank, the protocol of stimulation, or the IVF indication. Women were first informed by physicians and gave their written consent to participate in the study. Women who did not speak or read French and those refusing to participate were excluded. After embryo transfer, quantitative hCG was performed 14 days later and, if positive, a transvaginal ultrasound was performed at 8 weeks' gestation. Treatment with intravaginal natural progesterone (200 mg twice a day) was started from the day of oocyte retrieval to positive hCG blood test if pregnancy evolved.

Clinical data collected in medical files included age, body mass index, medical history, and gynecological history, in particular endometriosis, pelvic inflammatory diseases, tubal or ovarian surgery, familial history of hormone-dependent cancer or early menopause, duration of infertility, cause of infertility (male, female, joint, or idiopathic), prenatal drug exposure, birth weight, ovarian reserve markers (serum FSH, LH, E2, AMH levels, and ultrasonographic count of antral follicles on day 3), characteristics of IVF (IVF or ICSI, rank of the attempt, type of protocol used for ovarian stimulation, total dose of gonadotropin), and attempt results (biochemical pregnancy, clinical pregnancy, ectopic pregnancy, spontaneous miscarriage, medical termination of pregnancy, or live birth).

Biological data collected included estradiol level on the day of hCG administration, total number of oocytes collected, number of 2 PN zygotes, fertilization rate, number of diploid embryos, and number of embryos transferred.

Environmental exposure data collected via the self-administered questionnaire were smoking habits, alcohol or marijuana use, eating habits, parabens exposure, occupation at the time of IVF, and previous occupations. Each occupation was coded from the International Standard Classification of Profession–08 (ISCO-08). Armed force occupations were classified in group 0, managers in group 1, professionals in group 2, technicians and associates in group 3, clerical support workers in group 4, service and sales workers in group 5, skilled agricultural, forestry, and fishery workers in group 6, craft and related trades workers in group 7, plant and machine operators and assemblers in group 8, and elementary occupations in group 9.

The main outcome measure was clinical PR, defined as the presence of a gestational sac on ultrasonography at 8 weeks' gestation.

Statistical Analysis

Sample size was determined by a defined period of time and was not determined by power analysis. SPSS (Chicago, Illinois, USA) was used for statistical analysis. The results were expressed as mean ± standard deviation. The χ^2 test and Fisher's exact test were used to compare qualitative variables. Student's test was used to compare quantitative

variables. A multivariate logistic regression analysis was performed secondly. The significance level was set at p=0.05.

Results

Description of Study Population

During the study period, 386 women and 534 IVF starting cycles were included, with a cancellation rate of 7.6% for inadequate ovarian response to controlled ovarian stimulation (n=41). The mean age of patients was 33±5 years. Among the 386 women, 48% (n=186) reported being nonsmokers, 23% (n=89) were smokers, and 29% (n=111) were passive smokers. Regular cannabis use was reported by 2% (n=8) of women, and 49% (n=191) reported regular alcohol consumption (10 at 900 g per month with an average of 28±67 g per month). Characteristics of our study population were similar into the 2 groups and are summarized in Table I.

A total of 493 oocyte retrievals were performed, with 8 no-mature oocyte retrievals. Implantation rate per cycle was 26.4% (n=141), and clinical PR per cycle was 24% (n=129), with 82% (n=106) of ongoing pregnancy after 12 gestational weeks, 18% (n=22) of spontaneous miscarriage, and 1 legal termination of pregnancy for trisomy 21. Of the 106 clinical ongoing pregnancies, 88% (n=94) were singletons and 12% (n=12) were twins. Live birth rate per cycle was 19.8% (n=106) with 118 newborns: 51 girls and 67 boys.

Clinical Factors Influencing Pregnancy Rate

Women who achieved clinical pregnancy after embryo transfer were significantly younger than nonpregnant women (32.2±4.9 years vs. 33.8±5.1 years; p=0.002) and presented a significantly shorter period of infertility (3.5±1.9 years vs. 4±2.5 years; p=0.013) (Table II). Markers of ovarian reserve were not statistically different in the 2 groups (Table I). Concerning controlled ovarian stimulation protocol, the PR was significantly lower with the short protocol (15.7%) as compared to with the antagonist protocol (27.6%) and long agonist protocol (30.6%, p=0.003). All the other clinical characteristics were similar in the 2 groups.

Biological Factors Influencing Pregnancy Rate

Women who achieved pregnancy had a significantly higher number of mature oocytes retrieved (8.95±4.78 vs. 7.62±5.27), fertilization rate (69%±22% vs. 53%±32%), number of diploid embryos (4.83±2.95 vs. 3.47±3.22), and number of embryos transferred as compared with nonpregnant women (p=0.010, p<0.001, p<0.001, and p=0.021, respectively). Other biological factors were not statistically different and are summarized in Table II.

Environmental Factors Influencing Pregnancy Rate

Occupational Exposure (Table III). In our study population, 24% of women (n=91) were unemployed. After multivariate analysis, women whose occu-

Table I *Impact of Clinical and IVF Factors on Clinical Pregnancy Rate in IVF**

	No pregnancy	Clinical pregnancy	p Value
Age	33.8±5.1	32.2±4.9	0.002
BMI	24±4.7	24.1±4.5	NS
No. of years of infertility	4±2.5	3.5±1.9	0.013
Cause of infertility, no. (%)			NS
Female	92 (23)	28 (21)	
Male	176 (44)	63 (48)	
Mixed	78 (19)	27 (20)	
Idiopathic	59 (14)	11 (9)	
FSH level at cycle day 3 (IU/L)	7.3±2.3	7.2±2.3	NS
LH level at cycle day 3 (IU/L)	6.1±7.5	6.3±5.6	NS
E2 at cycle day 3 (IU /L)	49.9±40	44±22	NS
AMH, ng/mL	2.9±2.8	3.5±4.9	NS
Ultrasonographic count of antral follicles at cycle day 3	12±7	13±6	NS

*Results are expressed as mean±standard deviation.

Table II *Impact of Biological Factors on Clinical Pregnancy Rate in IVF*

Biological factors studied	No pregnancy No. (%)	Clinical pregnancy No. (%)	p Value
Classical IVF	244 (60)	73 (57)	NS
ICSI	161 (40)	56 (43)	
Range of IVF attempt			NS
T1	195 (48)	66 (51)	
T2	110 (27)	31 (24.5)	
>T2	100 (25)	31 (24.5)	
Level of E2 (pg/mL) the day of ovulation triggering	2,225±1,131	2,181±1,000	NS
No. of oocytes retrieved	7.62±5.27	8.95±4.78	0.010
No. of diploid embryos obtained at 48 h	3.47±3.22	4.83±2.95	<0.001
Fertilization rate	0.56±0.33	0.69±0.23	<0.001
No. of embryos transferred			0.021
1	136 (34)	39 (30)	
≥2	191 (47)	91 (69)	

Table III *Socio-professional Category (ISCO-08) and Clinical Pregnancy Rate*

Professional category ISCO-08	No pregnancy No. (%)	Clinical pregnancy No. (%)	p Value
ISCO-0	4 (1)	0 (0)	NS
ISCO-1	4 (1)	5 (4)	NS
ISCO-2	49 (12)	13 (10)	NS
ISCO-3	85 (22)	29 (23)	NS
ISCO-4	47 (12)	20 (16)	NS
ISCO-5	74 (19)	24 (19)	NS
ISCO-6	0 (0)	0 (0)	NS
ISCO-7	0 (0)	1 (1)	NS
ISCO-8	0 (0)	0 (0)	NS
ISCO-9	32 (8)	7 (5.5)	0.020
Unemployed	100 (25)	27 (21.5)	NS

pation belonged to the large group 9 of the international classification ISCO-08 ("elementary" occupations) had a lower clinical PR (OR 5.6; 95% CI 1.3–23.7; p=0.020) as compared to those in the other large groups. Among them, 82% (n=32) were cleaners.

Lifestyle and Domestic Exposure (Table IV). Among the study population, 63% (n=243) did not know if they were using cosmetics with parabens. We did not observe significant influence of lifestyle or domestic exposure on PR.

Discussion

Our study showed the influence of clinical and biological factors on IVF PRs, such as woman's age, type of protocol used for ovarian stimulation, number of oocytes collected, number of diploid embryos obtained, fertilization rate, and number of embryos transferred. These factors are well-known, and Roseboom et al built a statistical model assessing the probability of pregnancy after fresh embryo transfer according to age, cause of infertility, number of embryos transferred, and the average morphology score.[5] Rhodes et al, in a study of 205 patients, found higher PRs with the increase of fertilization rates, number of embryos transferred, and use of ICSI, and lower PRs with older women and blood on the transfer catheter.[18] We chose to observe these parameters first to have a general idea of factors influencing PRs in our population of IVF, but we will not discuss them in this article because there are no controversies about their influence.

The aim of this study was also to conduct a preliminary study of the impact of occupational, lifestyle, and domestic exposures on IVF PRs. Few studies have assessed these parameters in IVF, and only tobacco smoke is recognized by the scientific community as a toxic environmental factor in IVF, reducing PR and live birth after embryo transfer.[3] Regarding lifestyle behaviors, none of the factors studied (diet, cosmetic, physical activity, caffeine, alcohol, or marijuana consumption) significantly influenced the clinical PR in our study population. In the literature we found some contradictory studies. Homan et al, in a systematic review, found a negative impact of age, weight, and smoking, but they concluded that the evidence for the other factors is equivocal and needs further research.[19] Nicolau et al, in a systematic review of prospective studies, found a significant decrease in live birth rate in women who consumed at least 4 drinks per week (OR 0.84, 2,908 couples).[20] Palomba et al, in a case control study, found that regular physical activity carried out before an assisted reproduction cycle is significantly related with improved reproductive performance in obese, infertile patients, irrespective of bodyweight loss (clinical pregnancy and live birth: 3.22 (95% CI 1.53–6.78; p=0.002) and 3.71 (95% CI 1.51–9.11; p=0.004).[21] We think that our study cohort was probably too small to show an impact of lifestyle behaviors on IVF PR,

Table IV *Impact of Lifestyle Behaviors and Environmental Toxins on IVF Pregnancy Rate*

Lifestyle and domestic exposure	No pregnancy No. (%)	Clinical pregnancy No. (%)	p Value
Smoking status			NS
Tobacco smoke	96 (23)	36 (28)	
Passive tobacco	128 (32)	33 (25.5)	
Nonsmoker	180 (45)	60 (46.5)	
Active alcohol consumption	207 (52)	69 (54)	NS
Quantity of alcohol (g per month)	29±67	31±67	NS
Consumption of marijuana	7 (2)	4 (3	NS
Consumption of biological food	119 (29.5)	39 (30)	NS
Consumption of fish per month	3.6±2.7	3.9±3.2	NS
Coffee, no. of cups per day	1.8±1.7	1.9±1.7	NS
Caffeinated soda, no. of glasses per week	1.5±4	1.7±4	NS
Use of cosmetics containing parabens	18 (4.5) 39 (10)	7 (5 19 (14)	NS
Feeling stressed	344 (85)	110 (85)	NS

especially because exposition is often multiple and heterogeneous.

However, despite a small patient group, we found a significant decrease in PR among women working a job classified into group 9 of the ISCO-08, with 82% of those being categorized as "cleaners." This result must be interpreted carefully because our sample was small, but it leads us to an interesting questioning about a socio-professional category already recognized as at risk for congenital malformations during pregnancy.[22,23] Two hypotheses may be advanced: a potentially lower socioeconomic status and a theoretical increased exposure time to solvents. Unfortunately, we did not dispose of specific exposure biomarkers to assess the link between professional exposure to solvents and IVF PR. Baker et al, in a multivariate study on 225,889 cycles with fresh embryo transfer, found that a significant decrease of clinical PR in Blacks, Asians, and Hispanics may be due to lower socioeconomic status of these populations in the U.S.[6] We know that "cleaners" are exposed to many chemical substances found in cleaning products, like solvents, which are responsible for adverse health effects.[24] In the Pelagie study, "cleaners" had higher urinary levels of 2 metabolites of glycol ethers (ethoxyacetic acid and ethoxyethoxyacetic acid) than did those in other occupations.[25]

To our knowledge, there is no published study assessing the link between socio-professional category and IVF PRs. Koh et al reported that women who had been exposed to a cleaning solvent composed mainly of 2-bromopropane developed primary ovarian failure.[15] Prolonged times to pregnancy and increasing number of miscarriages have been observed in women exposed to solvents.[26] Chen et al, in a retrospective study conducted in female workers exposed to ethylene glycol ethers in semiconductor manufacturing, reported that women who were potentially exposed to ethylene glycol ethers showed longer time to pregnancy as compared with those not exposed (FR=0.59; 95% CI 0.37–0.94).[27] Sallmen et al showed reduced fertility among 250 shoe manufacturing workers exposed to organic solvents as compared with those not exposed, with a fecundability density ratio at 0.55 (CI 0.40–0.74) for low exposure and at 0.70 (CI 0.52–0.94) for high exposure.[28] The link between solvent exposure and lower PR in our study is only a hypothesis because we did not conduct a targeted inquiry on the type of handled products and the length of exposure. Although there are many confounding factors in our study, our objective was to conduct a preliminary and global study in order to select interesting environmental factors that need to be further studied in IVF.

In conclusion, IVF PR after embryo transfer depends on many biological, clinical, and environmental factors. The link between lifestyle behaviors and environmental factors remains theoretical and is difficult to prove because of the lack of specific biomarkers and because of a probably daily exposure to multiple toxic environmental agents with, furthermore, a possible synergistic effect of co-occurring agents.[29] However, despite our small sample size, pregnancy rates were significantly reduced in women working an "elementary" occupation. Further cohort studies are needed, and we project in the future to collaborate with the Occupational Medicine Department to assess the relationship between occupational exposure and fertility by using a specific interrogation, construction of a job exposure matrix, and biochemical tests to test solvent exposure in at-risk professions like hairdressers,[30] nail technicians,[31] and cleaners.[32]

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