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THE FERTILIZATION POTENTIAL OF DONOR SEMEN BETWEEN 1982 AND 2004 IN THE INDUSTRIAL AREA OF UPPER SILESIA (POLAND)

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Abstract: The industrial area of Upper Silesia is the most polluted region in Poland. To assess if these conditions could influence male fertility, a retrospective analysis of the fertilization potential of donor semen was performed, taking as an outcome measure the pregnancy rate after donor inseminations in 1982–2004. Data on contamination of air and soil in the region were collected and compared with those of the rest of the country. In total 2,100 inseminations using fresh semen from 44 healthy donors with proven fertility in 1,617 cycles in 290 infertile couples were performed in 1982–1995 and 2,010 inseminations using frozen semen from 20 healthy donors with proven fertility in 1,994 cycles in 414 infertile couples were performed in 1996–2004. Significantly higher values of air and soil pollution compared to the rest of the country were stated. Pregnancies occurred in 125 patients inseminated by fresh semen and in 85 patients inseminated by frozen banked semen. The insemination efficiency was lower than expected and a distinct declining trend was observed in both groups. Significant rise in the number of cycles necessary for achieving pregnancy was noted. The fertilization potential of fresh and frozen donor semen in Upper Silesia is low and seems still to be diminishing. It might be speculated that this phenomenon could be caused by the high degree of industrial pollution.

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INTRODUCTION

In recent years reports discussing the decline of semen quality in patients of fertility clinics as well as in healthy individuals and even semen donors have been published [1, 2, 9, 10, 14, 20, 24]. It is considered that this phenomenon might be caused by environmental factors [1, 17], mode of life or occupational exposure to fertility impairing factors [16]. Very interesting are the articles of Benoff *et al.* [3], who observed that the lead concentration in seminal plasma of sperm donors correlates with fertilization potential, and Wdowiak *et al.* [21] concerning the influence of electromagnetical waves on male fertility. All these reports, more or less pessimistic, mostly take into account the sperm volume, concentration, total sperm count and the motility or morphology of spermatozoa. The parameters mentioned above correlate with fertilization potential only to some extent [8, 11, 13, 15]. In essence, the ability to achieve a pregnancy must be considered the only true evidence of male fecundity. According to Thonneau *et al.* [18] the best measure of fertilization capacity of semen (i.e. if the man is “less or more fertile”) seems the time needed to achieve the pregnancy.

Since for decades the industrial area of Upper Silesia has been considered to be the most polluted region in Poland and one of the most polluted in Europe, we decided to perform a retrospective analysis to assess the fertilization potential of donor semen used for insemination taking as an outcome measure the conceptions in recipients.

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MATERIAL AND METHODS

Concentrations and amounts of the most important environmental air pollutants were calculated from selected data of yearly reports of the Central Statistical Office in Warsaw [6].

The first group of 290 infertile couples attending the clinics during the period 1982–1995 was treated by fresh donor semen. All of the women fulfilled the inclusion criteria: biphasic cycles, checked by basal body temperature (BBT), cervical mucus appearance and foliculometry from 1987, lack of any signs of an infection of all the genital tract, normal cervical smears and normal hysterosalpingogram. The couples lost in follow-up or those with ovulation disturbances, inflammation, anatomical anomalies or tubal factor were not included in the study. Twenty-two women, inseminated more than 12 cycles, which might testify to hidden fertility problems, were also excluded, although in this group three pregnancies also occurred. The mean age of the remaining 268 recipients in each year of the study at the moment of their first insemination is presented in Table 1.

Fresh specimens after liquefaction were used until 1995, but later, following the recommendations of the Polish Gynecological Society, only frozen semen was applied.

The second group consisted of 414 infertile couples fulfilling similar inclusion criteria and treated by frozen banked donor semen during the period 1996-2004. Fourteen women in this group inseminated more than 12 cycles, despite three pregnancies achieved, were also excluded. The mean age of the remaining 400 recipients in each year of the study at the moment of their first insemination is also presented in Table 1.

There are no significant differences between particular years in the population analyzed.

Indications for donor insemination are shown in Table 2. The indications are pooled in two parts: 1982–1995 (fresh semen) and 1996–2004 (frozen semen).

A significantly lower percent of male subfertility compared to azoospermia among indications for donor inseminations in the later period is present.

Table 2. Indications for donor insemination.

Indications	1982–1995 N (%)	1996–2004 N (%)
Azoospermia	171 (63.8%)	300 (75.0%)
Male subfertility	89 (33.2%)*	80 (20.0%)*
Other	8 (3.0%)	20 (5.0%)
Total	268 (100%)	400 (100%)

* $p<0.05$

The group of donors consisted of 64 healthy, exclusively married men, who had fathered at least one healthy baby not more than 2 years previously. All of them were inhabitants of the region of Upper Silesia, mostly non-smokers, students or young physicians, without any occupational exposure. None of them was older than 35 years and no one could be a carrier of any disease which might be transmitted to the offspring. No donor's wife had had an abortion. All the donors were examined in respect of sexually transmitted diseases and all had negative sperm cultures. The examinations were repeated every half year. No case of recipient infection by any pathogen present in specimen was noted.

Ejaculates were collected at the clinics after at least two days of sexual abstinence and the donors were paid. Each ejaculate was assessed according to WHO criteria [23]. Only those fulfilling the normal criteria were used for insemination, fresh (44 donors between 1982–1995) or freezing (20 donors between 1996–2004). The remaining ejaculates (only sporadic cases) were wasted.

Each donor participated in 42–82 inseminations (on average 64.2) during one to two years.

The treated couples as well as donors signed proper declarations and contracts.

Timing of insemination by fresh semen in 1,151 natural cycles depended on BBT, appearance of the cervical mucus and from 1985 on, sonographic examination. The insemination was performed when the BBT remained low, the cervical mucus was aqueous, transparent, stringy and the cervical orifice was widely open, and the diameter of

Table 1. Mean age and number of recipients at the time of first insemination in particular years.

Year	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Age	28.8	29.5	29.9	29.9	29.9	29.9	29.7	30.0	29.7	29.5	29.4	29.4
S.D.	±2.1	±2.4	±3.6	±3.6	±3.6	±3.8	±3.7	±5.2	±4.2	±4.2	±4.3	±4.4
(N)	3	9	12	9	16	13	14	22	15	11	29	34
Year	1994	1995		1996	1997	1998	1999	2000	2001	2002	2003	2004
Age	29.6	29.7		29.5	29.3	29.5	29.4	29.4	29.5	29.5	29.4	29.6
S.D.	±4.6	±4.7		±4.7	±4.7	±4.9	±4.8	±4.7	±4.6	±4.6	±4.6	±3.7
(N)	48	33		41	42	44	51	62	62	42	52	6

ovarian follicle exceeded 15 mm. If the BBT did not rise, the cervical mucus did not change and the follicle did not rupture, the insemination was sometimes repeated two days later. In 60 cycles stimulated by clomiphene citrate and one stimulated by human menopausal gonadotrophin (HMG), human chorionic gonadotrophin was administered when the leading follicle diameter was at least 18–19 mm and insemination was performed 24 hours thereafter. The procedure was sometimes repeated after 48 hours.

Insemination was performed in a dorsal lithotomy position. The cervix was cleaned in Cuzco speculum. The fresh semen (1 ml) was administered using a cervical cap. The cap was removed no later than after 24 hours.

Following the recommendations of the Polish Gynecological Society, the frozen semen was used after at least half a year of banking, when all the tests in respect of sexually transmitted diseases in donors were negative again.

Timing of insemination by frozen semen in 1,598 natural cycles depended mainly on transvaginal sonography. The insemination was performed when the diameter of ovarian follicle exceeded 15 mm and endometrium thickeness at least 6 mm. If the follicle did not rupture, the insemination was sometimes repeated two days later. In 63 cycles stimulated by clomiphene citrate and six stimulated by HMG, human chorionic gonadotrophin was administered when the leading follicle diameter was at least 19 mm and endometrium thickeness at least 6 mm. Insemination was performed 24 hours thereafter. The procedure was sometimes repeated after 48 hours.

Insemination was performed in a dorsal lithotomy position. The cervix was cleaned in Cuzco speculum. The thawed and washed semen (0.5 ml) was injected slowly into the uterine cavity using the Frydman catheter. After that the patient remained on the gynecological chair for about 5 minutes with the screw of the Cuzco speculum loosened to tighten the cervical canal. Thereafter the speculum was removed and patient got up.

Statistical analysis was performed using Statistica for Windows. The Pearson's coefficient r was calculated for each correlation using weighted data because of different numbers of patients treated in particular years of observation, and chi-square for comparison of pooled indications was used.

RESULTS

Data on yearly emission of pollutants by industrial properties comparing Upper Silesia and the rest of the territory of Poland are presented in Tables 3 and 4.

The percent of soil polluted by selected metals in II–IV degree was much higher in Upper Silesia in the year 1988 (zinc 16.82%, cadmium 21.5%, nickel 1.60% and lead 9.75%) than in the rest of Poland (zinc 1.20%, cadmium 1.17%, nickel 0.38% and lead 0.48%). The differences between all the values comparing Upper Silesia and the rest of the Polish territory mentioned above, as well as in Tables 3 and 4, are dramatically statistically significant.

During the observation period, 2,100 inseminations by fresh semen in 1,617 cycles in 210 patients were performed and thereafter 2,010 inseminations by frozen semen in 1,886 cycles in 414 patients. The distribution of those included in the study (1,531 inseminations by fresh semen in 1,212 cycles till 1995 and 1,784 inseminations by frozen semen in 1,667 cycles from 1996) is presented in Figure 1. The number of inseminations in particular years is always higher than number of cycles because of multiple inseminations performed in some cases.

In two patients, after fresh semen insemination, vaginitis developed and one of them aborted, probably because of keeping the cap inside for a long time – more than one month, and in one patient after insemination by frozen semen adnexitis developed.

During all the observation period 204 pregnancies were achieved (7.05% per cycle and 30.5% per patient). In the fresh semen insemination group 122 patients became pregnant. Two pregnancies were ectopic, 6 recipients aborted and 114 patients delivered healthy babies – one of them twins. The pregnancy rates of all the 268 patients – per patient and per cycle are shown in figures 2A and 3A respectively. In the

Table 3. Yearly emission of selected air pollutants by industrial properties (tons/km²).

Pollutant	Year	Upper Silesia	Poland (rest of the territory)
Industrial dust (total)	1972	81.9	5.49
	1978	95.2	5.91
	1984	67.6	4.70
	1988	61.3	4.41
Volatile ash	1972	41.2	3.70
	1978	58.4	3.98
	1984	41.1	3.49
	1988	39.7	3.25
Metallic dust	1972	11.4	0.32
	1978	12.3	0.37
	1984	13.5	1.27
	1988	7.1	0.16
Sulphur dioxide	1972	61.3	4.18
	1978	128.7	5.44
	1984	115.4	6.03
	1988	105.6	6.92
Nitrogen oxides	1972	0.6	0.17
	1978	2.4	0.41
	1984	26.6	1.48
	1988	32.6	1.80

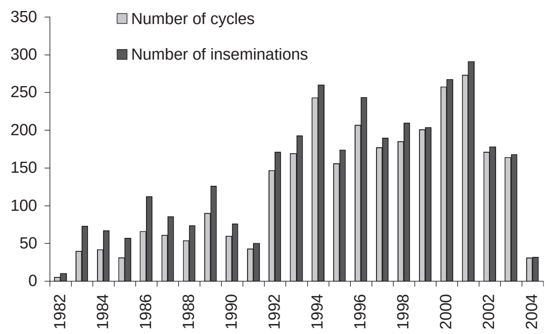

Figure 1. Number of cycles and inseminations in particular years 1982–2004.

frozen semen insemination group 82 pregnancies occurred. Two of them were ectopic, 8 patients aborted and 72 delivered healthy babies – among them two pairs of twins. The pregnancy rates of all the 400 patients – per patient and per cycle are shown in Figures 2B and 3B.

From the figures shown above (Figs. 2A, 2B, 3A, and 3B) it is clearly visible that in the period of fresh semen inseminations as well as in the period when only frozen semen was used, declining trends of conception rates are distinctly marked, to some extent less in the later group.

Figures 4A and 4B represent the mean number of insemination cycles needed for one conception in 122 patients who conceived in particular years 1982–1995 and in 82 patients who conceived in particular years 1996–2004, respectively.

In those figures (Figs. 4A and 4B) an evident rise in the number of insemination cycles needed for one conception (prolonged "time to pregnancy") is marked, especially in the later period.

Table 4. Yearly emission of selected metals by industrial properties (kg/km²).

Metal	Year	Upper Silesia	Poland (the rest of the territory)
Zinc	1995	20.1	0.13
	1999	4.00	0.057
	2002	4.79	0.0057
Cadmium	1995	0.25	0.0016
	1999	0.21	0.002
	2002	0.14	0.0036
Nickel	1995	0.17	0.018
	1999	0.026	0.0075
	2002	0.012	0.0059
Lead	1995	10.2	0.19
	1999	11.9	0.08
	2002	5.21	0.089

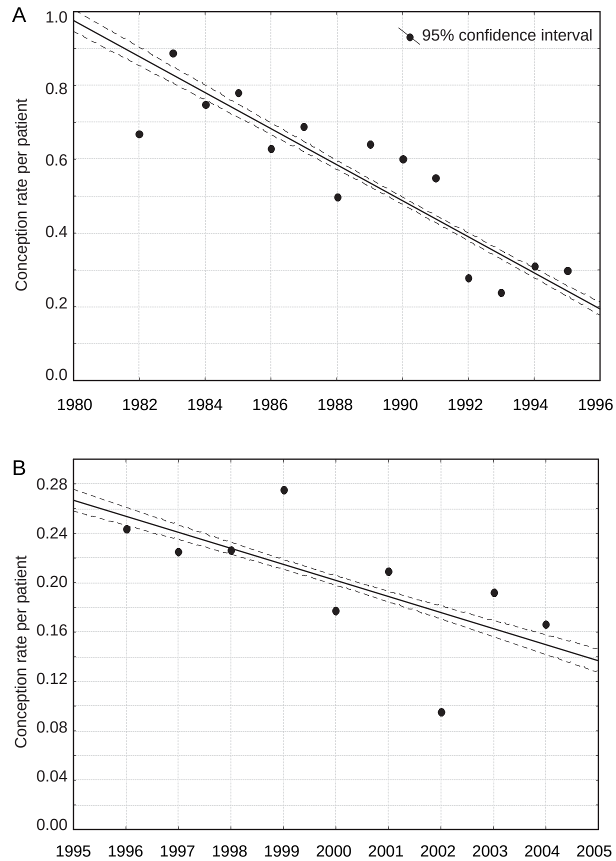

Figure 2. Mean conception rate per patient in the period: A) 1982–1995 (r = -0.9197, p<0.05); B) period 1996–2004 (r = -0.6064, p<0.05).

DISCUSSION

Opposite to assessment of routine parameters of the specimen, the evaluation of the fertilization capacity of the donor semen measured as the conception rate after its use is considered by some authors as the best method of estimation of the real donor fertility [19], because of hidden fertilization defects of spermatozoa.

Very low average efficiency value (7.09%) and clearly declining trends of the efficiency of donor inseminations were stated in both the periods analyzed. The average efficiency of donor insemination reported by Lamb [12] in the last decade of the last century is 10% per cycle.

The declining trends are more distinct, when counted per patient than per cycle or single procedure (Figs. 2 and 3). This might be caused by the stepwise lowering of the number of therapeutic cycles per one patient as well as a drop in inseminations per cycle. The limitation of the number of inseminations per cycle should not influence the results and is consistent with the opinion of Cantineau *et al.* [4], that the double insemination is not superior compared with a single insemination. The drop in the number of cycles per patient in turn was probably caused by introducing more advanced assisted reproduction techniques in our clinics. From that time, the recipients, left formerly only to inseminations untill

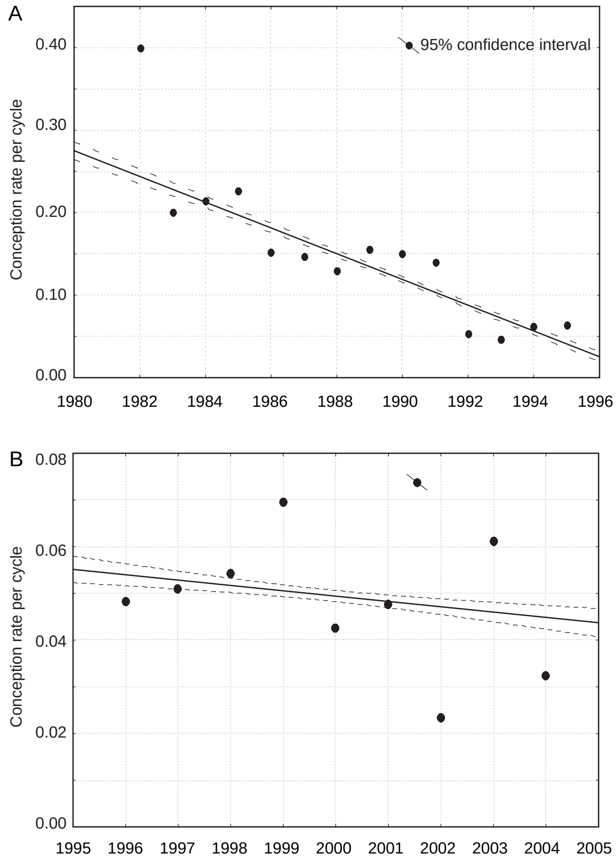

Figure 3. Mean conception rate per cycle in the period: A) 1982–1995 ($r = -0.8937$, $p<0.05$); B) 1996–2004 ($r = -0.2055$, $p<0.05$).

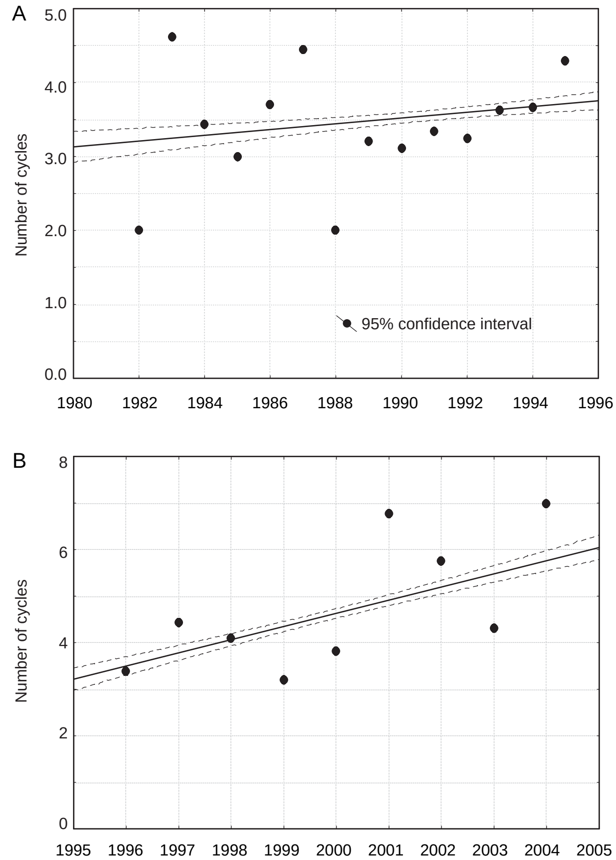

Figure 4. Mean number of cycles needed for one conception in women which became pregnant after donor inseminations by fresh semen noted in the period: A) 1982–1995 ($r = 0.24508$, $p < 0.05$); B) 1996–2004 ($r = 0.52065$, $p < 0.05$).

the conception or resignation because of loss of patience, might, after a few negative attempts, take an opportunity to be treated by a more effective method and finish inseminations. Thus, it is not unlikely that if they should continue treatment by inseminations, some of them might become pregnant after more cycles. It might be speculated that the rise in the number of insemination cycles needed to achieve pregnancy during the observation period (Fig. 4) should be more reliable. The overall pregnancy rates were lower in the later series than in the earlier one, despite a higher percent of azoospermia among indications, because many of the couples suffering from male subfertility dropped out of the study for intracytoplasmic sperm injection (ICSI). It is well known that spouses of azoospermic men are more frequently fertile than those who are oligoasthenoterratospermic (OAT) [7] because of spontaneous pregnancies occurring in hypothetic OAT group. Of course, the fertilizing potential of frozen semen is lower than that of fresh semen, but according to more recent data [22] the freezing does not impair the fertilization capacity of healthy donor semen, when up-to-date technologies are applied. Nevertheless, the declining trends in success rates during inseminations in 1996–2004 are also distinct.

The most important factor limiting female fertility is age, but all of our recipients were of the same mean age through the period analyzed. The pathology which might possibly impair the fertility of recipients was excluded during selection of patients for analysis. Moreover, taking into account only the patients fertile without any doubt (122 and 82, who conceived, Fig. 4), even more insemination cycles were needed later than formerly to achieve a pregnancy.

There are, of course, other factors influencing the results of inseminations: proper timing and technical performance of the procedure and fertility potential of the recipient [7].

The timing and performance of the procedure according to accepted criteria were the same in all cases and might, at most, be stepwise improved. Nevertheless, no improvement but a worsening of the insemination results was observed.

Upper Silesia is dramatically polluted compared with the rest of Poland [6]. Additionally, it should be speculated that although the emission of metallic pollutants has declined during recent years, they are more intrusive to humans because of acid rains caused by incomparably higher emission of sulphur dioxide and nitrogen oxides. Paasch *et al.* [14], comparing spermiograms in men born in the polluted Leipzig area between 1960–1970, also observed a worsening of sperm parameters, suggesting gonadotoxic action of pollutants during the intrauterine period of life of the male fetuses. Carrell *et al.* [5] and Younglai *et al.* [24], in turn, stated big unexplained differences of semen quality between many banks. From the data obtained we may conclude that the fertility of the donors in the region of Upper Silesia declined during the observation periods. Because the donors were not exposed to any known harmful occupational factors, it might be speculated that this phenomenon is caused by the pollution of the Silesian environment, the highest in Poland.

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