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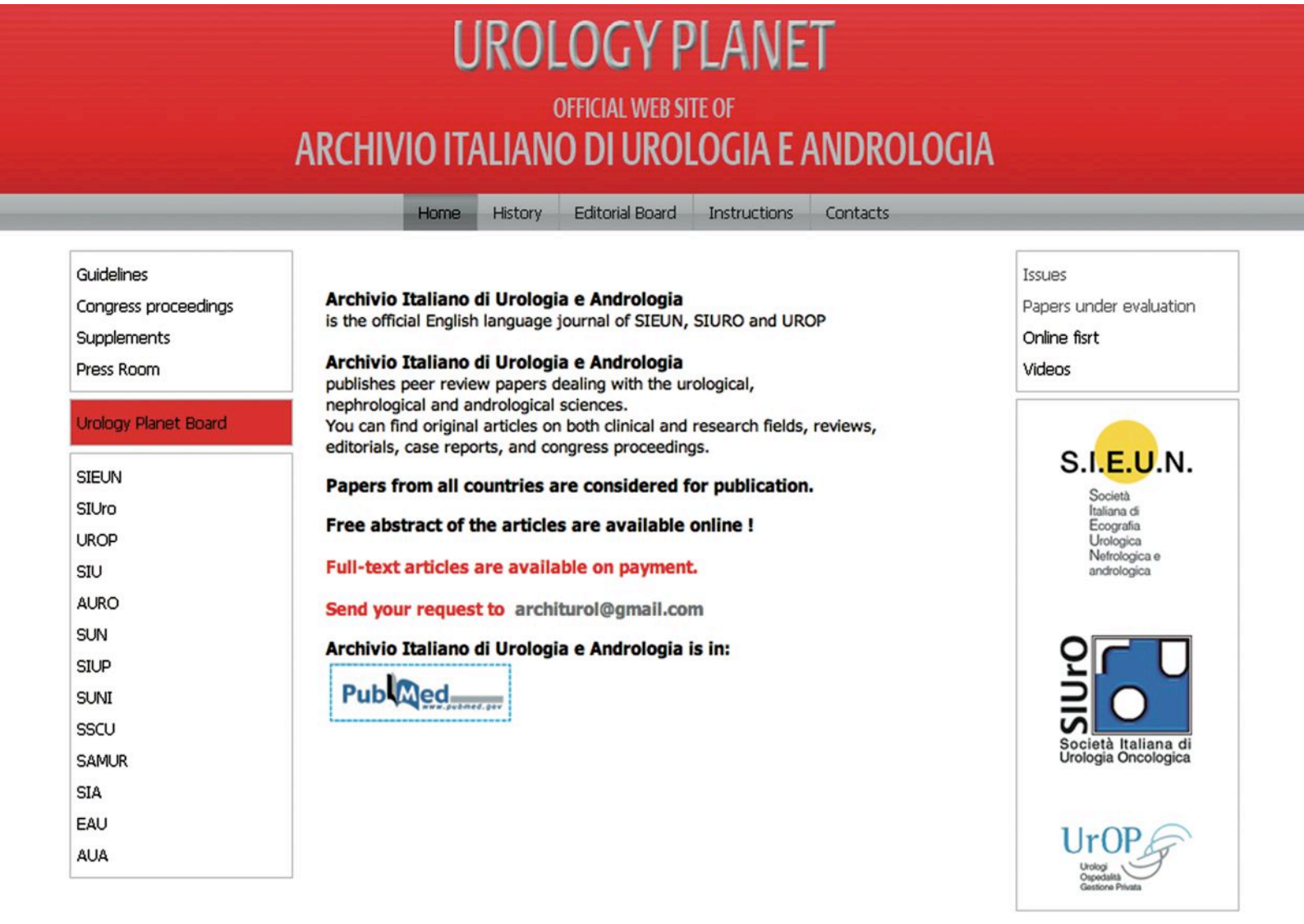

ORIGINAL PAPER

Comparative study of seminal parameters between samples collected in 1992 and samples collected in 2010

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Summary

Objective: *The aim of this study was to conduct a comparative study of semen quality in two large populations; one evaluated in 1992 and another in 2010, in order to evaluate any possible decline in male fertility due, at least in part, to environmental factors.*
Material and Methods: *A total of 701 subjects in 1992 (TOTAL group 1992) and a total of 626 subjects in 2010 (TOTAL group 2010) were enrolled in our Andrology Unit. Each group was subdivided into 3 subgroups: Subfertile, Pathology and Control.*
Standard semen analysis was performed using the Superimposed Image Analysis System, according to WHO guidelines 1987 (for TOTAL group 1992) and WHO guidelines 1999 (for TOTAL group 2010).
Results: *The mean values of sperm number (concentration/ml as well as the total ejaculate) and progressive motility were significantly higher in TOTAL group 2010 than TOTAL group 1992. Atypical forms in TOTAL group 1992 semen samples were significantly lower than TOTAL group 2010. The mean age of TOTAL Group 2010 was significantly higher compared with TOTAL Group 1992. In particular, the mean age gap was more evident in Subfertile subjects.*
Conclusions: *In conclusion, environmental factors have not determined a significant decline in seminal parameters in the past 18 years.*

KEY WORDS: *Environmental impact; Semen parameters; Standard semen analysis; Subfertility.*

Submitted 27 June 2011; Accepted 30 September 2011

INTRODUCTION

The increase in couple infertility seen in recent years is often ascribed to a decline in male fertilizing potential. The presumed reduction in the quality of seminal parameters has been ascribed not only to the increase in andrological diseases, but also to lifestyle (distress, smoking, alcohol, drugs). In the last two decades, environmental factors have also been hypothesized as having an impact on male fertility. Such factors are environmental chemical pollutants, for example heavy metals, pesticides, phthalates, etc. and physical factors, such as high temperatures, radiation, radio frequency electromagnetic radiation, and so forth.
The rationale for taking seminal parameters as a marker of the effect of such environmentally polluting factors is the fact that spermatogenetic function, owing to the continuous replication, differentiation and maturation of gametes, represents a useful model to study early damage caused by various toxic agents.
The first report in the literature regarding this hypothesis dates back to 1962, with "*Silent spring*" by *Rachel Carson*, who foresaw an apocalyptic scenario regarding the future of male fertility (1).
However, the substantive scientific debate on the decline in male fertility began in 1992, when *Carlsen et al.* (2) published a meta-analysis of 61 scientific works published from 1940 to 1990. This study showed a significant decrease in ejaculate volume and sperm number.
In 1997, *Colborn et al.* published "*Our Stolen Future*" (3), a new S.O.S. regarding human fertility. In this work, male fertility was seen as being under attack by chemical substances with estrogen-like effects in the environment.

Table 1.

Age and seminal parameters (Mean ± SD, Student t test, Mean difference and Confidence Interval) in the TOTAL groups and in the subgroups. p < 0.05 was considered significant.

	Groups and subgroups	1992 subjects n =	2010 subjects n =	Mean ± SD 1992	Mean ± SD 2010	Sig. (2-tailed)	Mean difference	95% Confidence interval of the difference	
Age (years)	**TOTAL**	701	626	32.8 ± 7.4	34.8 ± 8.6	0.000	-1.98	-2.86	-1.10
	Subfertile	440	303	34.5 ± 5.6	38.0 ± 7.0	0.000	-3.51	-4.45	-2.57
	Control	52	71	31.0 ± 8.0	35.4 ± 7.1	0.003	-4.36	-7.18	-1.55
	Pathology	209	252	29.9 ± 9.4	30.9 ± 9.3	ns	-0.97	-2.70	0.75
Volume (ml)	**TOTAL**	701	626	3.1 ± 1.6	3.2 ± 1.7	ns	-0.91	-2.97	-0.88
	Subfertile	440	303	3.3 ± 1.6	3.2 ± 1.6	ns	0.97	-1.41	0.33
	Control	52	71	2.8 ± 1.4	3.0 ± 1.5	ns	-0.11	-0.64	0.42
	Pathology	209	252	2.7 ± 1.6	3.2 ± 1.8	0.002	-0.51	-0.82	-0.19
Sperm number ($x10^6$/ml)	**TOTAL**	701	626	31.7 ± 24.9	57.4 ± 35.2	0.000	-25.66	-28.91	-22.33
	Subfertile	440	303	25.2 ± 22.8	55.0 ± 36.9	0.000	-29.75	-34.44	-25.06
	Control	52	71	42.8 ± 22.1	59.5 ± 35.7	0.002	-16.73	-27.16	-6.30
	Pathology	209	252	42.7 ± 24.9	59.7 ± 32.9	0.000	-17.01	-22.95	-11.73
Sperm number/ ejaculate ($x10^6$/ejac.)	**TOTAL**	701	626	98.4 ± 91.5	179.6 ± 146.8	0.000	-81.19	-94.56	-67.83
	Subfertile	440	303	87.2 ± 83.6	173.8 ± 152.9	0.000	-86.61	-105.57	-67.65
	Control	52	71	114.5 ± 69.6	169.5 ± 122.4	0.002	-55.00	-89.66	-20.23
	Pathology	209	252	118.0 ± 107.0	189.5 ± 145.6	0.000	-71.42	-94.55	-48.30
Progressive motility (%)	**TOTAL**	657	609	25.8 ± 16.3	29.7 ± 17.4	0.000	-3.95	-5.81	-2.08
	Subfertile	40	293	21.9 ± 15.5	27.1 ± 17.2	0.000	-5.28	-7.77	-2.79
	Control	51	70	30.7 ± 14.7	31.4 ± 16.6	ns	-0.66	-6.39	5.08
	Pathology	205	246	32.2 ± 16.0	32.3 ± 17.4	ns	-0.16	-3.24	2.93
Atypical forms (%)	**TOTAL**	657	609	53.9 ± 14.7	63.5 ± 12.9	0.000	-9.64	-11.16	-8.13
	Subfertile	40	293	57.3 ± 15.9	65.4 ± 13.9	0.000	-8.12	-10.35	-5.87
	Control	51	70	47.5 ± 6.9	62.2 ± 10.4	0.000	-14.67	-17.82	-11.52
	Pathology	205	246	48.8 ± 11.4	61.6 ± 12.1	0.000	-12.86	-15.04	-10.69
WBC ($x10^6$/ml)	**TOTAL**	701	626	1.0 ± 0.7	0.9 ± 0.9	ns	-0.84	-0.00	-0.17
	Subfertile	440	303	0.9 ± 0.7	0.9 ± 0.9	ns	0.12	-0.11	0.13
	Control	52	71	1.2 ± 0.9	0.9± 0.7	0.041	0.30	0.13	0.60
	Pathology	209	252	1.1 ± 0.7	0.9 ± 0.9	0.026	0.17	0.20	0.32

Table 2.

Seminal parameters subdivided into classes in the TOTAL groups and in the subgroups. In the Control subgroups, the classes have been grouped in order to perform statistical analysis (Chi-square test).

		TOTAL 1992 (n = 701) vs 2010 (n = 626) p < 0,0001		**Subfertile 1992 (n = 440-62.8%) vs 2010 (n = 303-8.4%) p < 0.0001**		**Pathology 1992 (n = 209-29.8%) vs 2010 (n = 252-0.3%) p = 0.767**		**Control 1992 (n = 52-7.4%) vs 2010 (n = 71-11.3%) p = 0.054**	
Sperm number ($x10^6$/ml)	**Azoospermia**	44 (6.3%)	17 (2.7%)	39 (8.9%)	10 (3.3%)	4 (1.9%)	6 (2.4%)	7 (13.4%)	3 (4.2%)
	< 5	75 (10.7%)	35 (5.6%)	63 (14.3%)	24 (7.9%)	11 (5.2%)	10 (4.0%)		
	≥ 5 < 15	78 (11.1%)	39 (6.2%)	66 (15.0%)	24 (7.9%)	10 (4.7%)	14 (5.6%)		
	≥ 15 < 20	60 (8.6%)	26 (4.2%)	43 (9.8%)	15 (5.0%)	14 (6.7%)	11 (4.3%)		
	≥ 20	444 (63.3%)	509 (81.3%)	229 (52.0%)	230 (75.9%)	170 (81.5%)	211 (83.7%)	45 (86.6%)	68 (95.8%)
		1992 (n = 657) vs 2010 (n = 609) p < 0.0001		**1992 (n = 401-61.0%) vs 2010 (n = 293-8.1%) p < 0.0001**		**1992 (n = 205-31.2%) vs 2010 (n = 246-0.4%) p = 0.186**		**1992 (n = 51-7.8%) vs 2010 (n = 70-11.5%) p = 0.595**	
Progressive motility (%)	**Immotility**	53 (8.1%)	23 (3.7%)	47 (11.7%)	13 (4.4%)	6 (2.9%)	9 (3.6%)	44 (87.8%)	59 (84.3%)
	< 5	6 (0.9%)	31 (5.1%)	4 (1.0%)	19 (6.5%)	2 (1.0%)	10 (4.1%)		
	≥ 5 < 32	370 (56.3%)	266 (43.7%)	252 (62.8%)	139 (47.4%)	91 (44.4%)	91 (37.0%)		
	≥ 32 < 50	161 (24.5%)	205 (33.7%)	72 (18.0%)	91 (31.1%)	71 (34.8%)	94 (38.2%)		
	≥ 50	67 (10.2%)	84 (13.8%)	26 (6.5%)	31 (10.6%)	35 (16.9%)	42 (17.1%)	7 (12.2%)	11 (15.7%)
		1992 (n = 657) vs 2010 (n = 609) p < 0.0001		**1992 (n = 401-61.0%) vs 2010 (n = 293-8.1%) p < 0.001**		**1992 (n = 205- 31.2%) vs 2010 (n = 246-40.4%) p < 0.0001**		**1992 (n = 51 - 7.8%) vs 1992 (n = 70 - 1.5%) p = 0.001**	
Atypical forms (%)	**< 50**	343 (52.2%)	60 (9.9%)	167 (41.6%)	26 (8.9%)	138 (67.3%)	29 (11.8%)	51 (100%)	57 (81.4%)
	≥ 50 < 70	237 (36.1%)	421 (69.1%)	167 (41.6%)	196 (66.9%)	57 (27.7%)	173 (70.3%)		
	≥ 70 < 96	42 (6.4%)	102 (16.7%)	37 (9.3%)	55 (18.7%)	5 (2.5%)	35 (14.2%)	0	13 (18.6%)
	≥ 96	35 (5.3%)	26 (4.3%)	30 (7.5%)	16 (5.5%)	5 (2.5%)	9 (3.7%)		

For this reason, these substances were called, "*hormone-disrupting chemicals*".
In the literature, there was a flood of studies, although not all in agreement, regarding the toxic effects of certain environmental chemical pollutants and physical factors on seminal parameters (4-8). However, in many of these works, the effects of each single factor were related to selected populations or occupational hazards (9-18).
Furthermore, a number of reports on the presumed variations in seminal parameters over time caused by environmental factors were in disagreement (19-25).
Therefore, the purpose of this work was to conduct a comparative study of the semen parameters of two large, homogeneous male populations in order to evaluate any possible decline in male fertility due, at least in part, to environmental factors. The first population had a standard semen analysis in 1992 and the second in 2010, thus allowing an interval of almost two decades to show the effects of any decline.

Materials and methods

Total subjects

The clinical study was conducted according to the *Hospital's Ethics Committee Guidelines*.
We carried out a comparative evaluation of seminal parameters on:

– n. 701 subjects (TOTAL group 1992) who had a standard semen analysis from January to December 1992;
– n. 626 subjects (TOTAL group 2010) who had a standard semen analysis from January to December 2010.

All the semen samples were collected by masturbation after 3-5 days of sexual abstinence.
After liquefaction, semen analysis in 1992 was carried out according to WHO guidelines 1987 (26) whereas in 2010 analysis was conducted according to WHO guidelines 1999 (27); in fact, we began to use the new WHO guidelines 2010 (28) last September.
We chose 1992 as our starting point because, in that year, our research group developed and started to use the Superimposed Image Analysis System (SIAS); this is a special software for computerized automatic/semiautomatic sperm motility assessment for objective semen analysis standardization (published in 1995) (29-30).
Each TOTAL group was subdivided into three subgroups based on the reason for referral for semen analysis:

- Subfertile: male partners of subfertile couples;
- Pathology: subjects affected by andrological pathologies such as varicocele, genital tract inflammation, previous trauma and funicular torsion, etc.;
- Control: subjects without clear symptoms and/or andrological pathologies, who requested a check up to evaluate their fertilizing capacity.

In all cases, where the subjects had more than one semen analysis during the study period, we considered only the first.

Statistical analysis

Results are expressed as percentages, mean values and standard deviations. Levene's test was used to assess the homogeneity of Variance. The Student t-test for independent samples was performed to compare the means of the two distibutions. The Pearson's chisquare test was performed to evaluate the association between qualitative variables and between seminal parameters subdivided into classes. $P < 0.05$ was considered significant.
All the statistical evaluations were performed using the SPSS program for Windows (release 13, 2004).

Results

1. Microscopic seminal parameters

The mean values ± SD and the statistical analysis of the TOTAL groups and the subgroups regarding all seminal parameters and ages are reported in Table 1. Figure 1 shows the mean values ± SD of sperm number, motility and morphology of the TOTAL groups in greater detail. Table 2 shows the results of the 3 subgroups according to the severity of the semen parameter alterations.

1.1 Sperm number

The means of sperm number and sperm number/ejaculate were significantly higher in the TOTAL group and in the subgroups 2010 compared with 1992 ($p < 0.001$) (Table 1; Figure 1). The prevalence of azoospermic and severe oligozoospermic subjects was clearly lower in TOTAL group 2010, as well as in Subfertile (Table 2).

1.2 Motility

The mean values of progressive motility were significantly higher in TOTAL group 2010 than TOTAL group 1992 ($p < 0.001$), as well as in Subfertile (Table 1; Figure 1). No significant differences were observed in Control (Table 1).
The prevalence of subjects with immotility was clearly lower in TOTAL group 2010 and in Subfertile (Table 2).

1.3 Morphology

The percentage of atypical sperm forms in the 1992 samples was significantly lower than in 2010 ($p < 0.001$) (Table 1; Figure 1). Regarding this semen parameter, the comparative data was less defined, owing to differences in the evaluation criteria of "normal" spermatozoa between WHO guidelines 1992 and 1999.

The prevalence of subjects in the subgroups considered as having atypical forms ≥96% is comparable in the 2 TOTAL groups 1992 and 2010 (Table 2).

2. Ejaculate volume

No significant differences were observed in the two TOTAL groups studied, or in Control or Subfertile subgroups (Table 1).

3. WBC

No significant differences were observed in the two TOTAL groups studied or in Subfertile (Table 1).
The mean values of WBC were significantly lower in Pathology and Control subgroups 2010 ($p < 0.05$) compared with 1992 (Table 1).

4. Age

The mean age of subjects that had a semen analysis in 2010 was significantly higher compared with that of

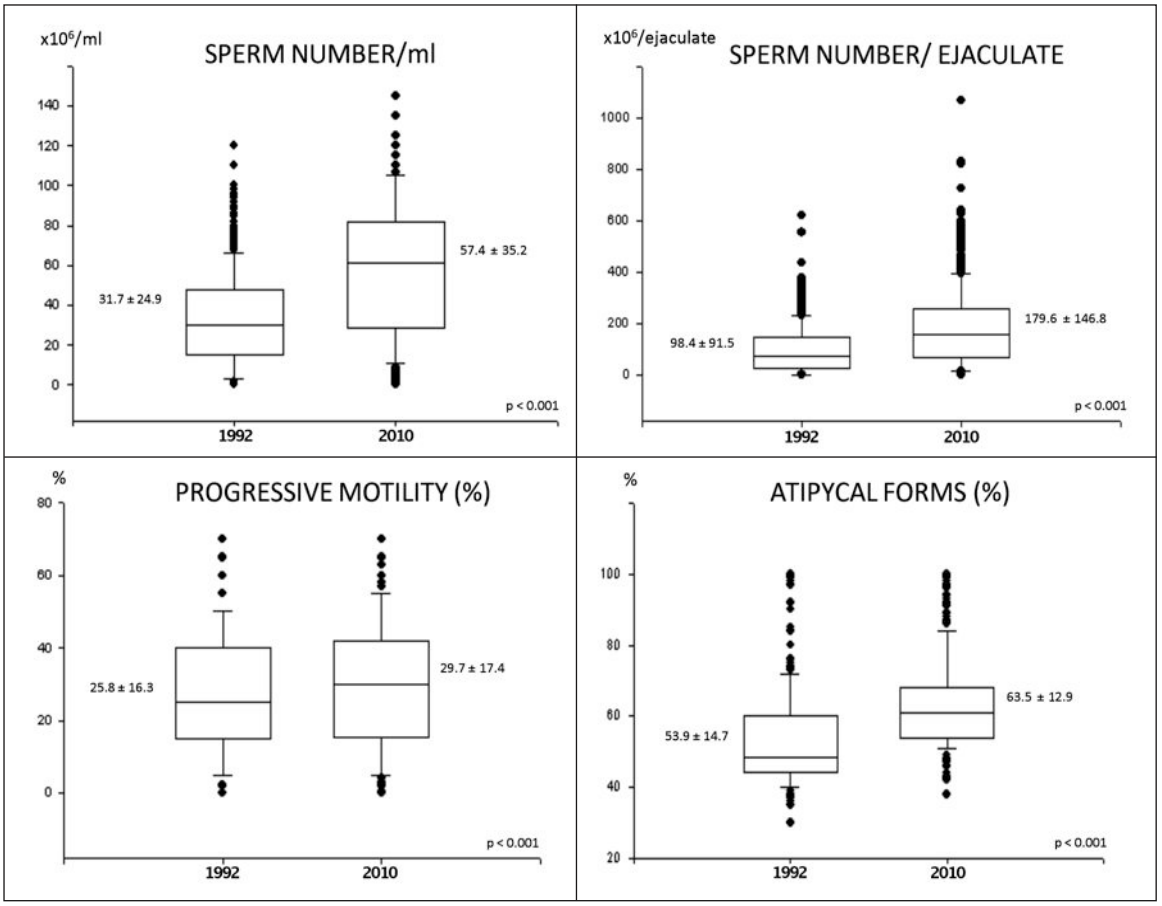

Figure 1.

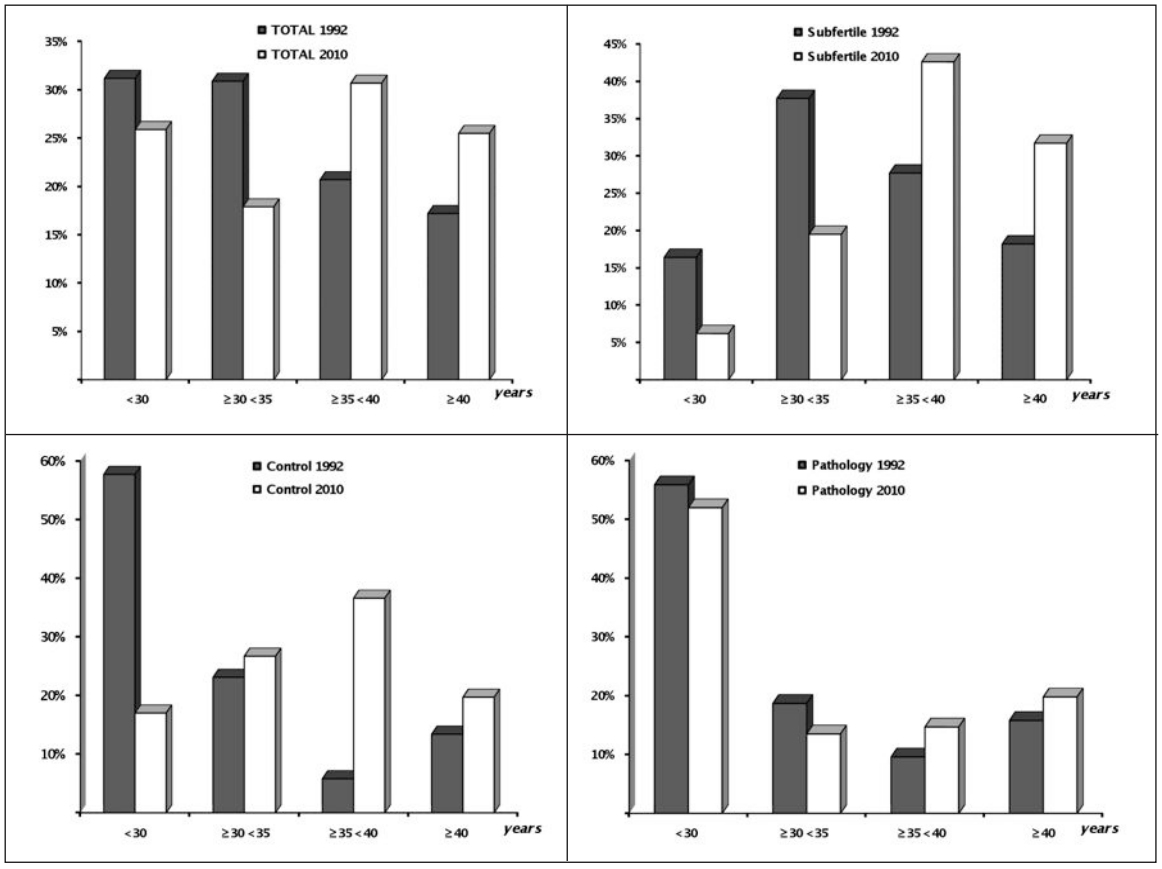

Figure 2.

1992 ($p < 0.001$). In particular, the mean age gap was more evident in Subfertile subjects (38.0 ± 7.0 years in 2010 vs 34.5 ± 5.6 years in 1992) (Table 1).
Furthermore, Figure 2 shows the percentage of the subjects in TOTAL groups and in the 3 subgroups according to age.

Discussion

The increase in the prevalence of couple subfertility has been attributed, at least in part, to the decline in male fertilizing ability. This presumed decline is thought to be due not only to an increase in classic andrological diseases and unhealthy lifestyle, but also to the negative impact of chemicals in the environment, such as pesticides, food additives, and phthalates. Physical factors are also thought to have a negative effect; examples are high temperatures and radiofrequency electromagnetic waves from cellular phones.
Therefore, this study focused on two large male populations that underwent semen analysis more than a decade apart and it served to highlight certain surprising details.
Regarding the real or presumed reduction in ejaculate volume, always considered by man as "*vulnus*" of his own virility, the study found that there were no significant differences between the two male populations.
Concerning the other seminal parameters, the mean values of sperm number (number/ml as well as number/ejaculate, which better reflects sperm production) and progressive motility were significantly higher in TOTAL group 2010 than TOTAL group 1992. In contrast, atypical sperm form percentages in TOTAL group 1992 semen samples were significantly lower than TOTAL group 2010. However, for this seminal parameter, the effective comparison of the data was complicated by changes made in the criteria used to define "*normal*" in the WHO guidelines 1999 compared with WHO guidelines 1987.
Furthermore, the presumed increase in WBC in the ejaculate, which is an expression of genital tract inflammation and/or infections, was not found in our study. Our data are partially in agreement with what has been observed by other authors (22-25); however, they do not show a correlation between environmental factors and a decline in seminal parameters. It may be that the blood-testicular barrier is able to defend the testicles.
Our results are strongly in disagreement with *Lackner et al.* (21) regarding the apocalyptic scenario used to describe subfertile subjects in 2003. The mean sperm concentration reported there was 4 mill/ml (excluding azoospermic subjects) compared with subfertile subjects in 1986, where the mean sperm concentration found was 27.5 mill/ml (excluding azoospermic subjects).
The undoubted increase in the prevalence of subfertile couples and the consequent reduction in birth rates could be the result of other factors, such as life-style, stress-related erectile dysfunction, the more advanced age of modern couples planning to have children, since "*socio-economic*" reasons have tended to delay procreation. In fact, this study highlights a significant age gap between Subfertile subjects 2010 and Subfertile subjects 1992; naturally, this difference is likely to be more relevant in female infertility.
In conclusion, even though this study has shown no deterioration in seminal profiles due to environmental factors, it is essential that society does not to lower its guard, since such environmental factors can, nonetheless, provoke toxic effects that can damage other organs and pose a risk to human health.

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