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Geographical differences in semen quality in a population of young healthy volunteers from the different regions of Spain

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Summary

Numerous studies have shown geographical differences in semen quality even within a given country. We have previously reported a low semen quality in volunteers from the province of Barcelona compared with the general population. The objective of this study was to determine the semen quality in a population of young healthy volunteers from the different regions of Spain. A total of 1239 volunteers between 18 and 30 years of age were enrolled. The parameters evaluated were semen volume, sperm concentration and progressive sperm motility. The results indicate that while there were no differences in semen volume or sperm motility, there were statistically significant differences in the rate of oligospermia in volunteers from the different regions studied. The prevalence of oligospermia was highest in Valencia (22.7%), Barcelona (22.7%) and Pais Vasco (18.7%), which are the regions of Spain with the highest degree of industrialisation for the last 50 years, and lowest in Galicia (8.5%) and Andalucía (13.7%), regions with a more recent industrial development. There were no differences in the rate of oligospermia as a function of age. These results support the hypothesis that exposure to environmental toxicants may affect the process of spermatogenesis leading to meiotic alterations, maturational arrest and oligospermia.

Introduction

Secular studies on semen quality have provided conflicting evidence suggesting in some studies that sperm concentration has declined significantly (Carlsen *et al.*, 1992; Auger *et al.*, 1995; Irvine *et al.*, 1996; Van Waeleghem *et al.*, 1996), whereas others have found no evidence of any change (Fisch & Goluboff, 1996; Paulsen *et al.*, 1996; Vierula *et al.*, 1996; Swan *et al.*, 1997). Perhaps the explanation for these conflicting results resides in regional differences in semen quality, which are at least as great as the possible secular trend. The possibility that these regional differences in sperm concentration may be biologically meaningful has been suggested by the observation of higher fertility in Finland than in the UK, when assessed by time to pregnancy, a functional measure of fertility (Joffe, 1996). However, it was found that couple fertility had increased in the UK during the period 1961–1993, and this may have compensated for a possible decline in male fertility (Joffe, 2000).

The regional differences and adverse trends seen in male reproductive health clearly involve aspects other than spermatogenesis. It is generally agreed that the incidence of testicular germ-cell cancer in adults is increasing and also shows great geographical variation (Adami *et al.*, 1994; Forman & Møller, 1994). Furthermore, congenital malformations of the male genital tract, such as hypospadias and cryptorchidism may have increased in some geographical regions (Ansell *et al.*, 1992; World Health Organization, 1999), although valid data are only available from very few areas.

Previous studies have shown that Danish men may have lower sperm concentration (Bostofte *et al.*, 1983) compared with Finnish men, who have high and

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unchanged sperm concentration (Vierula *et al.*, 1996). Furthermore, sperm concentration of French and Scottish (Auger *et al.*, 1995; Irvine *et al.*, 1996) and German (Paasch *et al.*, 2008) men seem to have declined in recent years.

Although endocrine disrupters have been proposed as one of the possible causes (Toppari *et al.*, 1996; Skakkebæk *et al.*, 2001), the topic remains controversial and the adverse trend is not apparent everywhere (Suominen & Vierula, 1993; Bujan *et al.*, 1996; Fisch & Goluboff, 1996; Paulsen *et al.*, 1996; Vierula *et al.*, 1996). Testicular cancer represents another aspect of male reproductive health, and in the Nordic–Baltic area there is a remarkable east–west gradient in the incidence of this disease. Finland and Estonia have a very low testicular cancer incidence which is less than one-third of that in Denmark and Norway (Adami *et al.*, 1994).

It was shown that young men from the general Danish population had a surprisingly low sperm concentration (median 45 million ml^{-1}) (Andersen *et al.*, 2000). Based on retrospective and not directly comparable data, Finnish men have previously been reported to have a high and unchanged mean sperm concentration (94–114 million ml^{-1}) (Suominen & Vierula, 1993; Vierula *et al.*, 1996).

Recent studies indicated that sperm concentration does not appear to change as a function of age (Eskenazi *et al.*, 2003; Lopez-Teijon, 2004). This suggests that the relatively low sperm concentration found in some geographical areas could be related to exposure to toxic factors, such as endocrine disruptors during the prenatal period in highly industrialised areas (Lopez-Teijon, 2004). Endocrine disruptors could induce alterations in the germ-cell line leading to alterations of meiosis, maturational block during spermatogenesis and a subsequent reduction in sperm concentration. If this damage were produced after puberty, one would expect to find worsening of sperm concentration as a function of age. This is also supported by the report of Carlsen *et al.* (1992) who did not find statistically significant differences in sperm concentration in a group of 158 young Danish adolescents studied over a period of 4 years. A study by Zheng *et al.* (1997) concluded that the decrease in sperm concentration observed in the Danish population corresponds to males born after 1950 and not before, suggesting that after 1950 a significant increase in the exposure to toxic environmental factors could explain, at least in part, the observed decrease in semen quality. These results are in good agreement with those reported by Andersen *et al.* (2000) who found a significant decrease in semen quality in a population of adolescents. These findings are consistent with an environmental effect during the prenatal period, but do not rule out the possibility that the putative testicular damage induced by environmental toxicants, such as endocrine disruptors, would not occur during the postnatal period before adolescence. However, if this were the case, it would not explain the increase in the incidence of congenital testicular anomalies observed in the last decades. Exposure to endocrine disruptors during the foetal period would be correlated with a higher prevalence of congenital anomalies such as hipospady, testicular cancer and cryptorchidism, which are all manifestations of the so-called testicular dysgenesis syndrome (Boisen *et al.*, 2001; Skakkebaek, 2002, 2003). Perhaps the decrease in sperm concentration could be another manifestation of this syndrome.

The main objectives of this study were: (i) to evaluate semen quality in volunteers from the different provinces and autonomous regions of Spain; and (ii) to determine what percentage of these males have normal semen parameters according to the WHO criteria.

Materials and methods

Study population

From July 1 to September 30 of 2007, 1239 volunteers from the different regions of Spain (both urban and rural population) were enrolled in the study. The age of the males ranged between 18 and 30 years. The study was carried out under the auspices of the Spanish Society of Andrology, the National Association of Assisted Reproduction Clinics and the Spanish Fertility Society. Volunteers were recruited through two main mechanisms: (i) a recruitment campaign to enrol patients was organised through a web page specifically designed for the study (http://www.hoynotelopongas.com), coupled to a press conference to disseminate through the media the objectives of the study and to raise awareness in the country about the significance of the study. A total of 548 volunteers from 42 provinces of Spain participated through the web page; and (ii) a total of 691 candidates to donor sperm of 10 different sperm banks in Spain were also enrolled in the study.

Study design

The study carried out in the 548 volunteers recruited through the web page was prospective, while the study with the candidates to donor sperm was retrospective. These males were asked to complete a questionnaire concerning their health status and lifestyle. Each volunteer provided a semen sample. Age, semen volume, sperm concentration and a + b motility were evaluated. Only healthy volunteers were enrolled in the study.

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Semen sample collection

Volunteers participating in this study were asked to provide a semen sample obtained by masturbation after an abstinence period of 3–4 days. The semen samples were collected into a wide-mouth, sterile, polypropylene container and delivered to the corresponding centre within 1 h after collection. Volunteers recruited through the web page delivered the semen sample to the participating centre nearest to their place of residence. Volunteers were asked to fill a validated questionnaire built into the web page that included body weight and height to calculate the body mass index, previous treatments (chemotherapy and radiotherapy), cryptorchidy, testicular surgery, sexually transmitted diseases, testicular traumatisms, hormonal treatments (e.g. anabolic steroids), toxic habits (tobacco and alcohol consumption), drug abuse, including type and frequency, sexual habits. The same applied to the sperm banks, as part of the routine donor screening. Therefore, only healthy volunteers were enrolled. A total of 61 centres participated through the web page recruitment mode.

Semen analysis

Semen analysis was performed by well-trained technicians from the different centres participating in the study. A total of 1239 semen samples were evaluated according to the WHO guidelines for semen analysis (World Health Organization, 1999). Semen parameters evaluated included ejaculate volume, sperm concentration and per cent a + b motility according to WHO criteria. Aliquots of the semen samples were analysed within 30–60 min after semen liquefaction.

Quality control

Eighty-five per cent of all centres participating in the study through the web page and all the sperm banks also participated in an external quality control system for semen analysis with Centro de Estudio e Investigación de la Fertilidad (CEIFER, Granada) in collaboration with the ESHRE and the Asociación para el Estudio de la Biología de la Reproducción.

Statistical analysis

Dichotomous variables (normal/abnormal) were generated to evaluate each of the semen parameters. According to the WHO guidelines, sperm concentration above 20 million ml^{-1}, semen volume $\geq$ 2 ml and per cent a + b motility above 50% were considered normal. A final composite variable was derived from those three variables only in the case that all three variables were normal.

Taking as a reference, the place of residence of the volunteer, the province or autonomous region was determined therefrom. To determine the semen quality, a descriptive study was carried out at three different levels:

1. Only for participants with residence in Spain and Spanish nationality.

2. Taking into account the mode of recruitment of the volunteers.

3. Analysis by province and autonomous regions.

The objective of the first level was to offer a global vision of the semen quality in volunteers from Spain. The second level allows assessing whether the two different modes of volunteer recruitment had any effect on the results (web page versus sperm banks). Lastly, the third level will allow detecting significant differences in semen quality in the different geographical areas of Spain.

A power analysis was conducted to detect 25% differences in the prevalence of the semen parameters evaluated in the different geographical areas, with a confidence interval of 95% and a power of 85%. Using these criteria, the minimal sample size of volunteers required for the different geographical regions was of 70.

Differences in semen parameters as a function of the mode of recruitment of the volunteers were evaluated using chi-squared test. Differences in semen quality in the different autonomous regions were evaluated using analysis of variance and *post hoc* multicomparison tests (T2 Tamhane).

The statistical analysis of the levels previously described was performed in two ways: using the information about the semen characteristics from the quantitative variables 3 obtained in the study and using the categorisation of these variables where semen quality was indicated as normal or abnormal.

4 The software used was SPSS version 15.0.1.

Results

The seminal characteristics of semen volume, per cent sperm motility and sperm concentration are shown in Tables 1–8. All variables were treated in a quantitative manner and categorised as a function of whether they were normal or abnormal.

The mean semen volume was 3.69 ml with a standard deviation 1.65 ml. Seventy-five per cent of the volunteers had a semen volume lower than or equal to 4.7 ml and the remaining 25% a volume above this value (Table 1).

The mean per cent motility observed in the overall volunteer population evaluated was 51.04% with a typical standard deviation of 17.69%. Seventy-five per cent of the volunteers presented a motility equal to or below 62% and the remaining 25% had a per cent motility above this value (Table 1).

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Table 1 Semen parameters in healthy young volunteers

	Motility (%)	Volume (ml)	Concentration ($\times 10^6$ ml^{-1})
Mean	51.04	3.69	68.82
Standard deviation	17.69	1.65	54.60
Minimal	0	0.05	0
Maximal	95	11	750
Percentile 25	40	2.5	32
Median	51	3.4	60
Percentile75	62	4.7	90
n	1239	1239	1239

Table 2 Semen parameters and semen quality in healthy young volunteers (abnormal/normal)

	n	%
Volume		
Abnormal	122	9.8
Normal	1117	90.2
Motility		
Abnormal	612	49.4
Normal	627	50.6
Concentration		
Abnormal	217	17.5
Normal	1022	82.5
Semen quality[a]		
Abnormal	716	57.8
Normal	523	42.2
Total	1239	100.0

[a]Normal semen quality includes normal volume, motility and concentration.

The mean sperm concentration observed was 68.82 million ml^{-1} with a standard deviation of 54.60 million ml^{-1}, an important degree of variability. Seventy-five per cent of the volunteers had a concentration equal to or below 90.0 million ml^{-1} and the remaining 25% was above this value (Table 1).

The proportion of volunteers with normal and abnormal sperm motility was the same (50%). The great majority of the participants had a normal semen volume (90.2%) and normal concentration (82.5%) (Table 2). When the three parameters evaluated were considered, 57.8% of the participants had normal semen parameters.

The semen parameters obtained through the two different recruitment modalities were very similar in both groups (Fig. 1). The *t*-test of equality of the means showed that there were no statistically significant differences in concentration or semen volume, but there were statistically significant differences in per cent motility (Tables 3 and 4). Both sources of data showed a similar proportion of normal semen quality.

When the prevalence of normal semen quality was evaluated using all three parameters, no statistically significant differences were found (Table 4). Hence, it can be concluded that the data obtained through both modes of recruitment are comparable and therefore can be combined.

The information corresponding to the different provinces and autonomous regions is shown in Tables 5 and 6 respectively. Some of the provinces and regions had a low number of participants recruited. Although descriptively, all data from the different geographical areas were incorporated; to carry out a statistical comparison and arrive to meaningful conclusions a power analysis was conducted (see *Materials and methods*) and only those autonomous regions with more than 70 participants were evaluated (Table 7).

The proportion of volunteers with a normal semen volume and normal sperm motility was highest in Galicia and Catalunya, while sperm concentration was highest in Galicia, Andalucía and Madrid (Table 7). Galicia was the autonomous region with the highest proportion of sperm concentration (91.5%), while Valencia and Catalunya had the lowest proportion of normal sperm concentration (77.3%). Galicia was also the autonomous region with the

Table 3 Semen parameters in healthy young volunteers (abnormal/normal) by information source

	'Hoy no te lo pongas'			Semen banks		
	Motility (%)	Volume (ml)	Concentration ($\times 10^6$ ml^{-1})	Motility (%)	Volume (ml)	Concentration ($\times 10^6$ ml^{-1})
Mean	50.89	3.86	68.63	51.15	3.56	68.98
Standard deviation	18.13	1.72	59.10	17.35	1.58	50.79
Minimum	0	0.3	0	0	0.05	0
Maximum	95	11	750	91	9.8	343.90
Percentile 25	40	2.5	30	40	2.5	34
Median	52	3.5	60	50	3.2	60
Percentile75	61	5	90	64	4.5	90
n	548	548	548	691	691	691

'Hoy no te lo pongas' refers to the web page through which volunteers were recruited.

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Table 4 Semen parameters and semen quality in healthy young volunteers (abnormal/normal) by information source

	'Hoy no te lo pongas'			Semen banks			
	n	%	CI 95%	*n*	%	CI 95%	*P*[a]
Volume							
Abnormal	46	8.4	6.1–10.7	76	11.0	8.7–13.3	0.126
Normal	502	91.6	89.3–93.9	615	89.0	86.7–91.3	
Motility							
Abnormal	252	46.0	41.8–50.2	360	52.1	48.4–55.8	0.033
Normal	296	54.0	49.8–58.2	331	47.9	44.2–51.6	
Concentration							
Abnormal	101	18.4	15.2–21.7	116	16.8	14.0–19.6	0.450
Normal	447	81.6	78.3–84.8	575	83.2	80.4–86.0	
Semen quality							
Abnormal	303	55.3	51.1–59.5	413	59.8	56.1–63.4	0.113
Normal	245	44.7	40.5–48.9	278	40.2	36.6–43.9	
Total	548	100.0		691	100.0		

[a]*P*-value, chi-squared test.
'Hoy no te lo pongas' refers to the web page through which volunteers were recruited.

highest proportion of normal semen quality (62.4%). If these six autonomous regions were to be evaluated using analysis of variance, these differences will be also confirmed for sperm concentration, semen volume and per cent a + b motility (Tables 7 and 8).

Finally, when the prevalence of oligospermia was evaluated, statistically significant differences were found in the different provinces and autonomous regions. As shown in Fig. 2 and Fig. 3, Galicia was the autonomous region with the lowest rate of oligospermia, followed by Andalucía, Asturias and Madrid, while Valencia, Catalunya and Pais Vasco were the autonomous regions with the highest rate of oligospermia. Interestingly enough, these last three autonomous regions, with the highest rate of oligospermia, are the ones with the highest degree of industrialisation for the last 50 years.

Discussion

The main findings emerging from this study are the significant geographical differences in semen quality observed in the different provinces and autonomous regions of Spain. Although two different modes of recruitment were utilised, the data obtained through these two databases were very similar, thus validating the merging of the data from these two sources.

One potential criticism that could be raised, particularly in the recruitment mode through the web page, would be the great heterogeneity in the reproducibility of the results in the 65 participating centres. However, the fact that the data obtained in the 10 participating sperm banks was highly homogeneous, that these centres follow a rigorous internal and external quality control programme and that almost identical results were obtained through both data sources, suggests that the data obtained through the participating centres of the web page can be considered valid. In fact, one of the reasons why sperm morphology was not included in the semen parameters to be evaluated was precisely the difficulty in obtaining meaningful results given the high heterogeneity in the morphology criteria used in different centres and their reproducibility.

An unexpected finding was the significant differences in the rate of oligospermia found in the autonomous regions of Galicia, Asturias, Andalucía, Madrid, Catalunya, Valencia and Pais Vasco. These autonomous regions make up a relatively high percentage of the population in Spain. Furthermore, the highest rate of oligospermia was found in those autonomous regions with the highest degree of industrialisation in the last 50 years. Although Galicia, Asturias, Madrid and Andalucía have experienced an increase in the degree of industrialisation, this has taken place in more recent years. The results reported by Paasch *et al.* (2008) are also in agreement with those described above, because a significant proportion of young men from Germany, a country with a high degree of industrialisation in the last 50 years, shows a sperm concentration below normal limits.

One potential limitation of this study could be the selection bias that may have occurred during the recruitment process. That is, the candidates to sperm donors who provided semen samples to the sperm banks or the volunteers who provided samples to the collaborating centres may not be representative of the general population of their corresponding provinces and regions because a 'self-selection' process was involved. However, although based on theoretical grounds this is a possibility, because the main objective of this study was to compare semen

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Table 5 Normal semen parameters in healthy young volunteers by province of residence

	% normal								
	n	Volume	CI 95%	Motility	CI 95%	Concentration	CI 95%	Semen quality[a]	CI 95%
Valencia	192	82.8	77.5–88.1	56.3	49.2–63.3	78.1	72.3–84.0	41.1	34.2–48.1
Granada	132	88.6	83.2–94.1	30.3	22.5–38.1	87.1	81.4–92.8	25.0	17.6–32.4
A Coruña	124	96.0	92.5–99.4	64.5	56.1–72.9	93.5	89.2–97.9	62.1	53.6–70.6
Barcelona	117	92.3	87.5–97.1	64.1	55.4–72.8	80.3	73.1–87.5	56.4	47.4–65.4
Madrid	108	90.7	85.3–96.2	41.7	32.4–51.0	85.2	78.5–91.9	37.0	27.9–46.1
Bizkaia	69	91.3	84.7–98.0	46.4	34.6–58.1	79.7	70.2–89.2	34.8	23.5–46.0
Asturias	57	94.7	88.9–100.0	75.4	64.3–86.6	80.7	70.5–90.9	59.6	46.9–72.4
Zaragoza	38	81.6	69.3–93.9	42.1	26.4–57.8	76.3	62.8–89.8	26.3	12.3–40.3
Valladolid	35	91.4	82.2–100.0	65.7	50.0–81.4	82.9	70.4–95.3	51.4	34.9–68.0
Sevilla	31	93.5	84.9–100.0	35.5	18.6–52.3	74.2	58.8–89.6	29.0	13.1–45.0
Malaga	29	96.6	89.9–100.0	41.4	23.5–59.3	93.1	83.9–100.0	37.9	20.3–55.6
Murcia	29	100.0	100.0–100.0	44.8	26.7–62.9	62.1	44.4–79.7	41.4	23.5–59.3
Alicante	24	83.3	68.4–98.2	45.8	25.9–65.8	83.3	68.4–98.2	33.3	14.5–52.2
Cordoba	24	83.3	68.4–98.2	33.3	14.5–52.2	95.8	87.8–100.0	29.2	11.0–47.4
Tarragona	21	95.2	86.1–100.0	52.4	31.0–73.7	57.1	36.0–78.3	42.9	21.7–64.0
Jaen	20	95.0	85.4–100.0	25.0	6.0–44.0	75.0	56.0–94.0	25.0	6.0–44.0
Cadiz	18	100.0	100.0–100.0	38.9	16.4–61.4	83.3	66.1–100.0	33.3	11.6–55.1
Pontevedra	17	94.1	82.9–100.0	70.6	48.9–92.2	76.5	56.3–96.6	64.7	42.0–87.4
Badajoz	16	100.0	100.0–100.0	75.0	53.8–96.2	87.5	71.3–100.0	68.8	46.0–91.5
Caceres	16	100.0	100.0–100.0	37.5	13.8–61.2	93.8	81.9–100.0	37.5	13.8–61.2
Leon	16	100.0	100.0–100.0	68.8	46.0–91.5	81.3	62.1–100.0	62.5	38.8–86.2
Las Palmas	12	91.7	76.0–100.0	41.7	13.8–69.6	75.0	50.5–99.5	33.3	6.7–60.0
Ciudad Real	11	63.6	35.2–92.1	36.4	7.9–64.8	72.7	46.4–99.0	27.3	1.0–53.6
Almeria	10	80.0	55.2–100.0	10.0	0.0–28.6	90.0	71.4–100.0	10.0	0.0–28.6
Santa Cruz de Tenerife	9	88.9	68.4–100.0	22.2	0.0–49.4	88.9	68.4–100.0	11.1	0.0–31.6
Huelva	7	85.7	59.8–100.0	42.9	6.2–79.5	100.0	100.0–100.0	28.6	0.0–62.0
Lleida	7	100.0	100.0–100.0	85.7	59.8–100.0	85.7	59.8–100.0	85.7	59.8–100.0
Guipuzkoa	6	100.0	100.0–100.0	50.0	10.0–90.0	100.0	100.0–100.0	50.0	10.0–90.0
Girona	5	100.0	100.0–100.0	20.0	0.0–55.1	80.0	44.9–100.0	20.0	0.0–55.1
Illes Balears	5	80.0	44.9–100.0	40.0	0.0–82.9	40.0	0.0–82.9	0.0	0.0–0.0
Navarra	5	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0
Segovia	5	80.0	44.9–100.0	20.0	0.0–55.1	100.0	100.0–100.0	20.0	0.0–55.1
Castellon	4	75.0	32.6–100.0	25.0	0.0–67.4	0.0	0.0–0.0	0.0	0.0–0.0
Guadalajara	4	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0
La Rioja	4	100.0	100.0–100.0	75.0	32.6–100.0	100.0	100.0–100.0	75.0	32.6–100.0
Cantabria	3	100.0	100.0–100.0	66.7	13.3–100.0	100.0	100.0–100.0	66.7	13.3–100.0
Huesca	3	33.3	0.0–86.7	33.3	0.0–86.7	66.7	13.3–100.0	0.0	0.0–0.0
Ceuta	2	50.0	0.0–100.0	0.0	0.0–0.0	100.0	100.0–100.0	0.0	0.0–0.0
Cuenca	1	0.0	0.0–0.0	100.0	100.0–100.0	100.0	100.0–100.0	0.0	0.0–0.0
Melilla	1	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0
Teruel	1	0.0	0.0–0.0	0.0	0.0–0.0	100.0	100.0–100.0	0.0	0.0–0.0
Toledo	1	100.0	100.0–100.0	0.0	0.0–0.0	100.0	100.0–100.0	0.0	0.0–0.0
Total	1239								

[a]Normal semen quality includes normal volume, motility and concentration.

quality in healthy volunteers from the different provinces and regions of Spain, one would expect that if a selection bias were to occur though this recruitment process, it would affect all provinces and regions in a similar fashion. As the rate of oligospermia in volunteers from those autonomous regions with an adequate recruitment sample size that were included in the final analysis, shows profound differences among these autonomous regions (Catalunya, Valencia, Pais Vasco versus Galicia, Andalucía, Madrid), it is highly unlikely that a selection bias could have had a significant impact on the results.

Despite the fact that a rigorous internal and external quality control programme was followed, it is possible that the variability observed in the results could be due, at least in part, to the heterogeneity in the protocols used by the different centres that participated in this study.

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Table 6 Semen parameters and semen quality by autonomous region of residence

	% normal								
	n	Volume	CI 95%	Motility	CI 95%	Concentration	CI 95%	Semen quality[a]	CI 95%
Andalucia	271	90.4	86.9–93.9	32.1	26.5–37.7	86.3	82.3–90.4	27.3	22.0–32.6
Comunidad Valenciana	220	82.7	77.7–87.7	54.5	48.0–61.1	77.3	71.7–82.8	39.5	33.1–46.0
Catalunya	150	93.3	89.3–97.3	62.0	54.2–69.8	77.3	70.6–84.0	54.7	46.7–62.6
Galicia	141	95.7	92.4–99.1	65.2	57.4–73.1	91.5	86.9–96.1	62.4	54.4–70.4
Madrid	108	90.7	85.3–96.2	41.7	32.4–51.0	85.2	78.5–91.9	37.0	27.9–46.1
Pais Vasco	75	92.0	85.9–98.1	46.7	35.4–58.0	81.3	72.5–90.2	36.0	25.1–46.9
Asturias	57	94.7	88.9–100.0	75.4	64.3–86.6	80.7	70.5–90.9	59.6	46.9–72.4
Castilla-Leon	56	92.9	86.1–99.6	62.5	49.8–75.2	83.9	74.3–93.5	51.8	38.7–64.9
Aragon	42	76.2	63.3–100.0	40.5	25.6–55.3	76.2	63.3–89.1	23.8	10.9–36.7
Extremadura	32	100.0	100.0–100.0	56.3	39.1–73.4	90.6	80.5–100.0	53.1	35.8–70.4
Murcia	29	100.0	100.0–100.0	44.8	26.7–62.9	62.1	44.4–100.0	41.4	23.5–59.3
Canarias	21	90.5	77.9–100.0	33.3	13.2–53.5	81.0	64.2–97.7	23.8	5.6–42.0
Castilla-La Mancha	17	70.6	48.9–92.2	52.9	29.2–76.7	82.4	64.2–100.0	41.2	17.8–64.6
Illes Balears	5	80.0	44.9–100.0	40.0	0.0–82.9	40.0	0.0–100.0	0.0	0.0–0.0
Navarra	5	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0
La Rioja	4	100.0	100.0–100.0	75.0	32.6–100.0	100.0	100.0–100.0	75.0	32.6–100.0
Cantabria	3	100.0	100.0–100.0	66.7	13.3–100.0	100.0	100.0–100.0	66.7	13.3–100.0
Ceuta	2	50.0	0.0–100.0	0.0	0.0–0.0	100.0	100.0–100.0	0.0	0.0–0.0
Melilla	1	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0	100.0	100.0–100.0

[a]Normal semen quality includes normal volume, motility and concentration.

Table 7 Semen parameters by autonomous region of residence (*n* > 70)

		% normal					
		Volume*		Motility*		Concentration*	
	n	Mean	SD	Mean	SD	Mean	SD
Andalucia	271	3.87	1.74	45.61	14.34	74.52	54.36
Comunidad Valenciana	220	3.21	1.49	52.76	17.06	49.16	29.62
Catalunya	150	3.90	1.67	53.62	20.05	70.43	57.36
Galicia	141	3,72	1.45	57.23	18.22	74.99	40.94
Madrid	108	3.56	1.51	45.80	16.66	85.36	86.87
Pais Vasco	75	3.47	1.70	45.64	14.43	53.12	30.04
Total	965						

**P*-value < 0.05, analysis of variance.

Table 8 Multiple comparisons in semen parameters by autonomous region of residence (*n* > 70)

	Andalucía	Catalunya	C. Valenciana	Galicia	Madrid	País Vasco
Andalucía		M	V C M	M		C
Catalunya	M		V C		M	C M
C. Valenciana	V C M	V C		V C	C M	M
Galicia	M		V C		M	C M
Madrid		M	C M	M		C
País Vasco	C	C M	M	C M	C	

V, volume; C, concentration; M, motility with *P*-value <0.01, multiple comparison (T2 Tamhane).

It is worth noting that the conclusions reached in a previous study, in which the sperm concentration of volunteers from the University of Almeria (autonomous region of Andalucía) was considered normal, as compared with other European countries (Avivar *et al.*, 2004), cannot be extrapolated to all provinces and autonomous regions of Spain. In fact, the results of this study in terms of sperm concentration reproduce those reported by

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Table 9 Normal semen parameters and normal semen quality by autonomous region of residence ($n > 70$)

	% normal								
	n	Volume	CI 95%	Motility	CI 95%	Concentration	CI 95%	Semen quality[a]	CI 95%
Andalucia	271	90.4	86.9–93.9	32.1	26.5–37.7	86.3	82.3–90.4	27.3	22.0–32.6
Comunidad Valenciana	220	82.7	77.7–87.7	54.5	48.0–61.1	77.3	71.7–82.8	39.5	33.1–46.0
Catalunya	150	93.3	89.3–97.3	62.0	54.2–69.8	77.3	70.6–84.0	54.7	46.7–62.6
Galicia	141	95.7	92.4–99.1	65.2	57.4–73.1	91.5	86.9–96.1	62.4	54.4–70.4
Madrid	108	90.7	85.3–96.2	41.7	32.4–51.0	85.2	78.5–91.9	37.0	27.9–46.1
Pais Vasco	75	92.0	85.9–98.1	46.7	35.4–58.0	81.3	72.5–90.2	36.0	25.1–46.9

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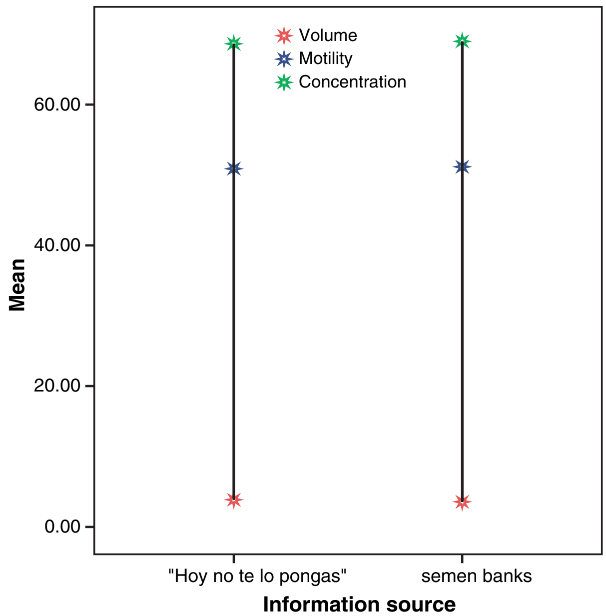

Fig. 1 Semen parameters in healthy young volunteers by information source.

Avivar *et al.* (2004) In that study, the rate of oligospermia was 14.5%, very close to the 13.7% obtained in our study. However, as shown in this study, Andalucía, together with Galicia, were the autonomous regions of Spain with the highest sperm concentration and the lowest rate of oligospermia of all the autonomous regions of Spain. In contrast, the rate of oligospermia of volunteers from Catalunya, the Pais Vasco and Valencia was significantly higher when compared with Andalucía. This, once again, stresses the importance of evaluating regional differences in semen quality, even within a given country.

Zheng *et al.* (1997) reported that a decrease in sperm concentration was observed in the Danish population to males born after 1950 and not before, suggesting that after 1950 a significant increase in the exposure to toxic environmental factors. This could explain, at least in part, the observed decrease in semen quality in the Danish population. These results are in good agreement with those reported by Andersen *et al.* (2000) who found a significant decrease in sperm concentration in a population of adolescents. These findings are consistent with an environmental effect during the prenatal period

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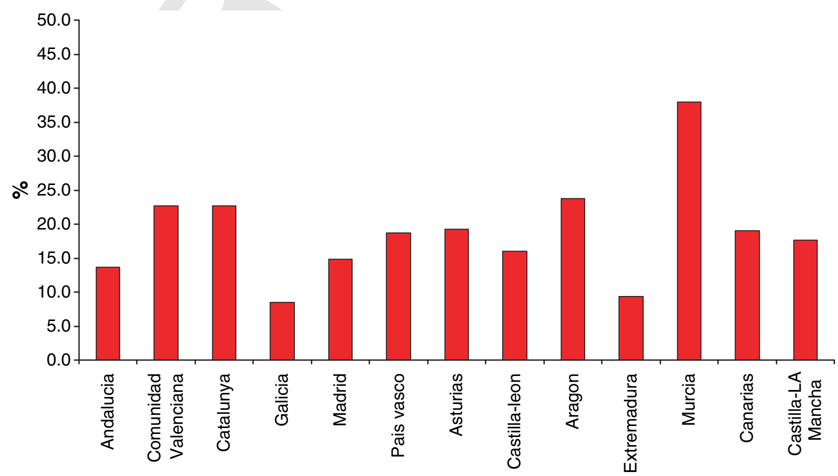

Fig. 2 Abnormal semen concentration by autonomous region of residence.

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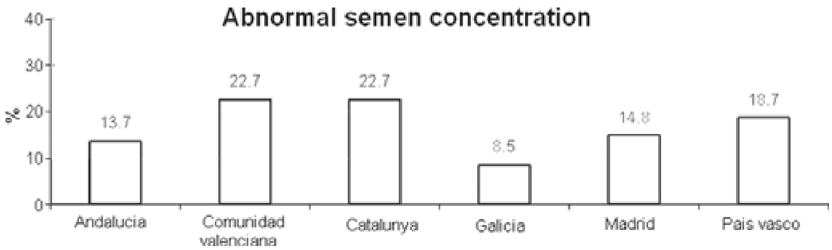

Fig. 3 Abnormal semen concentration by autonomous region of residence.

5 (Lopez-Teijon, 2004). However, as pointed out in the introduction, these results do not rule out the possibility that the putative testicular damage induced by environmental toxicants, such as endocrine disruptors, would not occur during the postnatal period before adolescence. However, if that were the case, this would not explain the increase in the incidence of congenital testicular anomalies observed in the last decades (Skakkebæk *et al.*, 2001). Exposure to endocrine disruptors during the foetal period would be correlated with a higher prevalence of congenital anomalies such as hipospady, testicular cancer and cryptorchidism, which are all manifestations of the so-called testicular dysgenesis syndrome (Boisen *et al.*, 2001; Skakkebaek, 2002, 2003). Perhaps the decrease in sperm concentration could be another manifestation of this syndrome. In a recent study by Shen *et al.* (2008), it was shown that the concentration of organochlorine compounds in human milk and placenta is significantly higher in samples obtained from Danish compared with Finnish women. It has previously been reported that a lower reproductive health status, including a higher rate of cryptorchidism and hypospadias, is observed in Danish compared Finnish males (Shen *et al.*, 2008). This further supports the hypothesis that prenatal exposure to endocrine disruptors may be a potential cause of impaired spermatogenesis and pre-disposition to testicular cancer.

In conclusion, the results of this study showed significant geographical differences in semen quality in volunteers from the different provinces and autonomous regions of Spain. Of particular interest were the significant differences in the rate of oligospermia observed, being highest in the more industrialised regions of Spain. These results support the hypothesis that exposure to environmental toxicants may affect the process of spermatogenesis leading to meiotic alterations, maturational arrest and oligospermia.

Studies are currently underway to compare the concentration of endocrine disruptors during pregnancy and lactation in women from those provinces and autonomous regions of Spain with significant differences in the rate of oligospermia.

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