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Have Sperm Counts Deteriorated Over the Past 20 Years in Healthy, Young Japanese Men? Results From the Sapporo Area

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ABSTRACT: Changes in semen quality of healthy men is a controversial issue throughout the world. It is suspected that many chemical endocrine disrupters may affect the quality of semen. Although exposure to them may be extensive in Japan, no evidence of changes in semen quality has been reported. In this study, changes in semen volume and sperm counts were analyzed over 20 years in the Sapporo area of Japan. Semen volume and sperm counts were measured in 254 and 457 normal, healthy volunteers who lived in the Sapporo area in 1975–1980 and 1998, respectively. Posters and handbills were used to recruit participants in both studies. Semen samples were collected by masturbation after 3 days or more of abstinence. There was no change in semen volume between 1975–1980 and 1998. Mean sperm counts were $70.9 \pm 47.3 \times 10^6$/mL in 1975–1980 and $79.6 \pm 49.3 \times 10^6$/mL in 1998. Sperm counts did not decline over about 20 years. No significant correlation between age and sperm counts was recognized in either study. The rates of subjects with oligozoospermia and azoospermia were the same in both studies. In the 1975–1980 study, 34 of 254 (13.4%) participants had a child, and in the 1998 study, 51 of 457 (11.2%) participants had a child. Mean sperm count was significantly ($P < .02$) lower in the earlier study ($66.0 \pm 44.9 \times 10^6$/mL) than in the 1998 study ($98.7 \pm 60.2 \times 10^6$/mL). This is the first reliable report in which changes in sperm counts in Japan were studied. We conclude that there was no evidence of deterioration in sperm counts of normal healthy men who lived in the Sapporo area of Japan over 20 years. However, selection bias in the recruitment of volunteers and the issue of variable abstinence might have affected the results of these studies. Therefore, well-designed prospective studies should be performed in several different regions to extrapolate our results on sperm counts to healthy, young Japanese men in general.

Key words: Fertility, endocrine disruptors, seminalysis.

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According to a meta-analysis of 61 papers, Carlsen et al (1992) reported that sperm counts were decreased by 50% over the past 50 years. Semen quality in healthy men was evaluated in many countries because endocrine-disrupting chemicals, in particular weak estrogenic chemicals that contaminate food, plant, and industrial materials, were suspected to be one of the causes of the impairment of male reproductive function (Sharpe and Skakkebaek, 1993). Many manuscripts about this issue have been published. Some of them reported evidence of decreasing sperm counts (Auger et al, 1995; Adamopoulos et al, 1996; Irvine et al, 1996; Joffe, 1996; Menchini-Fabris et al, 1996; Van Waeleghem et al, 1996; Younglai et al, 1998), whereas others reported that the count was steady or increased (Bujan et al, 1996; Fisch et al, 1996; Paulsen et al, 1996; Vierula et al, 1996; Rasmussen et al, 1997). The reasons for the discrepancies of these results in sperm counts found among the reports include study design, selection bias, methods used for measurement of sperm, regional differences, and the methods employed for statistical analysis.

In Japan, chronological deterioration of sperm counts in healthy men has been discussed for some years. Because Japan is one of most industrially advanced countries in the world, exposure to endocrine-disrupting chemicals during the fetal period has been a matter of concern (Saegusa, 1998). Moreover, the Japanese diet includes many weak estrogens that originate from grains, vegetables, and soybeans (Adlercreutz et al, 1991). It is suspected that these dietary estrogens may play a role in reducing the number of patients who suffer from prostate cancer, which is sensitive to androgens (Griffiths, 1996). Estrogen is believed to suppress spermatogenesis not only by a negative feedback mechanism in the secretion of gonadotropins from the pituitary gland, but also via a direct effect on the testis (Sharpe et al, 1998). Based on these findings, impairment of semen quality in Japanese men has been suspected; however, no reliable data about sperm counts have been reported in this country. Therefore, we considered it crucial to conduct an epidemiolog-

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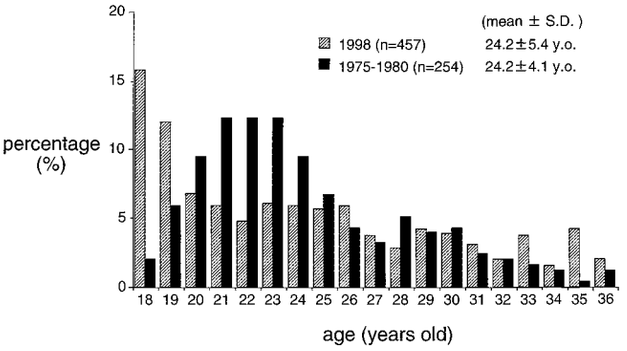

Figure 1. Age distributions of volunteers in 1975–1980 and 1998.

ical study of sperm counts in healthy, young Japanese men.

In this study, we evaluated sperm counts in 457 volunteers while reducing biases in selection of subjects and semen examination. Moreover, to assess the chronological change in sperm counts in healthy young men who lived in the Sapporo area over a period of about 20 years, the results of the current study were compared with those of a previous study that was performed in the same area during 1975 to 1980 at the same institution (Ikegaki et al, 1984).

Subjects and Methods

Subjects

A total of 457 healthy men aged 18 to 36 years participated in this study in 1998. All of them lived in the Sapporo area. Our department had already performed and reported the results of semen analysis in 254 male healthy volunteers between 1975 and 1980 (Ikegaki et al, 1984). In the 1998 study, volunteers were recruited through handbills that were distributed throughout town. All participants were informed that the objective was to investigate changes in sperm counts in the Sapporo area over the past 20 years. Volunteers agreed to provide semen samples for the current study. All volunteers were paid for their participation. In the 1975–1980 study, posters were used to recruit volunteers and no inducement was paid for participation. Volunteers were informed that the objective was to establish the normal range of sperm counts in healthy, young Japanese men. In both studies, participants who had suffered from genitourinary disorders or who had been treated for any diseases at the time of registration were excluded. Whether they were married or had a child were not criteria for participating in either study.

Analysis of Semen

Semen samples were collected by masturbation into wide-mouth sterile plastic cups at our university hospital after 3 days or more days of abstinence. When participants came to our hospital for seminalysis, the period of abstinence was confirmed to be 3 days or more; however, we did not ask about the exact number of days of abstinence. A single sample was collected from each volunteer. Samples were allowed to liquefy at room temperature for 20–30 minutes before analysis. Semen volume was determined by aspirating the liquefied sample into a 5-mL syringe with an 18-gauge needle. After measuring semen volume, 10 μL of gently mixed semen was added to 190 μL of diluting fluid containing 5% $NaHCO_3$ and 1% formalin (Wako Co, Ltd, Osaka, Japan). The number of sperm was counted using a Thoma's blood counting chamber (Kayagaki Works Co, Ltd, Tokyo, Japan) under a microscope at a final magnification of 400×. In both studies (1975–1980 and 1998), the entire process of analysis was performed in our laboratory. All of the procedures, including collection of semen, measurement of semen volume, and counting the number of sperm in the current study were performed exactly as in the earlier study based on an examination of the records and the manuscript (Ikegaki et al, 1984).

Table 1. *Seminal parameters of healthy Japanese men in 1975–1980 and 1998*

Study	No. of Subjects	Seminal Volume, mL*	Semen Density (×10⁶/mL) Mean*	Median
1975–1980	254	2.8 ± 1.2	70.9 ± 47.3	62
1998	457	2.9 ± 1.3	79.6 ± 49.3	75

* Values are expressed as the mean ± standard deviation.

Statistical Analysis

The Mann-Whitney *U*-test or analysis of variance was used to analyze all data. When the *P* value was .05 or less, the difference was defined as statistically significant.

Results

Age Distribution of Volunteers

Mean ages of volunteers were 24.2 ± 4.1 (mean ± SD) years in 1975–1980 and 24.2 ± 5.4 years in 1998. Although the mean age was almost the same in both studies, the distribution of age was slightly different (Figure 1). Peaks of age were 21–23 years in 1975–1980 and 18 years in 1998. As mentioned later, because sperm counts were not correlated with age in either study, complete matching of age distribution was not believed to be necessary for comparison of the data between the 2 studies.

Semen Volume and Sperm Counts in the Two Studies

Mean semen volumes were 2.8 ± 1.2 mL in 1975–1980 and 2.9 ± 1.3 mL in 1998. These values were not significantly different (Table 1). Mean sperm counts (× 10^6/mL) were 70.9 ± 47.3 in 1975–1980 and 79.6 ± 49.3 in 1998. No significant differences in sperm counts were recognized between the 2 studies (Table 1). Median sperm counts (× 10^6/mL) were 62 in 1975–1980 and 75 in 1998. These findings indicated that semen volume and sperm counts did not decline during the past 20 years.

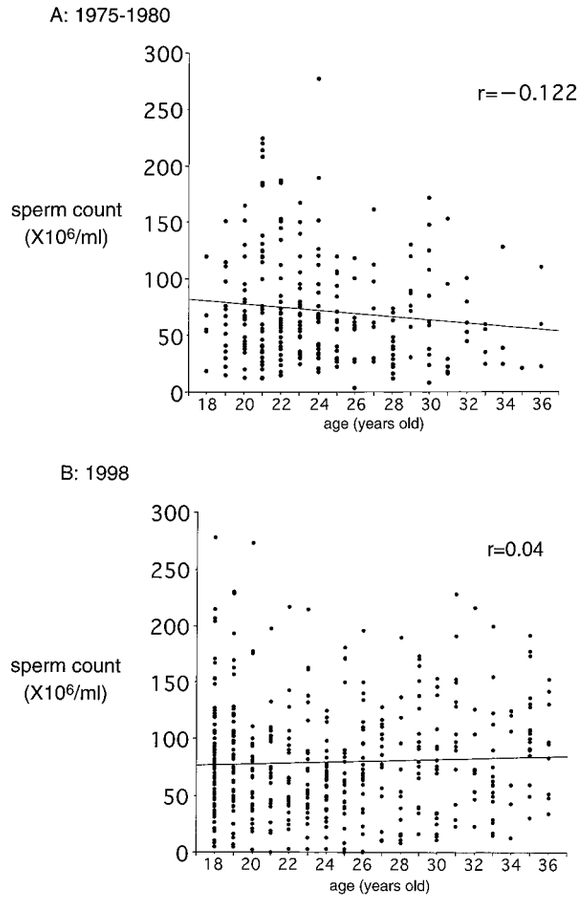

Figure 2. Relationships between age at donation and sperm density. Linear regression analysis showed no significant correlation between them in 1975–1980 and 1998. **(A)** 1975–1980; **(B)** 1998.

Age and Sperm Counts

The relationship between age and sperm counts was analyzed to elucidate the effect of aging on sperm counts. No obvious correlation was recognized between age and sperm counts in the 2 studies (Figure 2). This observation was the reason why complete age matching was not performed between participants of the 1975–1980 and 1998 studies, despite the slight difference in age distribution, when semen volume and sperm counts were compared between the 2 studies.

After participants were classified into 4 groups by age, no significant difference of averaged sperm counts was observed between groups (Table 2).

Percentage of Subjects With Azoospermia or Oligozoospermia

One of 254 (0.4%) and 2 of 457 (0.4%) subjects had azoospermia in the 1975–1980 and 1998 studies, respectively. Oligozoospermia, defined as a sperm counts of less than 20×10^6/mL (World Health Organization, 1999) were found in 17 of 254 (6.7%) participants in 1975–1980 and in 40 of 457 (8.8%) participants in 1998. No significant difference in percentages of subfertile subjects having abnormal sperm counts was recognized between the 2 studies.

Sperm Counts in Fertile Subjects

In the 1975–1980 study, 13.4% (34 of 254) of participants had a child, and 11.2% (51 of 457) of participants in the 1998 study had a child. In both studies, fertility was not a criterion for participation. Mean ages in these studies were not significantly different (30.7 ± 3.6 years in 1975–1980 and 30.1 ± 3.2 years in 1998. The mean sperm count of fertile subjects in the 1975–1980 study was significantly ($P < .02$) lower ($66.0 \pm 44.9 \times 10^6$/mL) than in 1998 ($98.7 \pm 60.2 \times 10^6$/mL).

Discussion

Deterioration of sperm counts and increases in the number of patients with other male reproductive disorders, such as cryptorchism, hypospadias, and testicular cancer have recently been reported (John Radcliffe Hospital Cryptorchidism Study Group, 1992; Adami et al, 1994; Matlai et al, 1985). Many endocrine-disrupting chemicals that are likely to act as estrogens or androgens are suspected to be possible candidates that may affect male reproductive disorders. These observations have led investigators to re-evaluate sperm counts in normal men in many countries. In particular, Japanese people may be exposed to industrial and dietary estrogens that could lead to a deterioration of sperm counts (Adlercreutz et al, 1991; Saegusa, 1998). To clarify the actual state of sperm

Table 2. *Sperm density according to age at donation*

	Age, y*			
Study	18–22	23–27	28–32	33–36
1975–1980 (n = 254)	80.0 ± 53.1 (n = 107)	70.9 ± 45.3 (n = 91)	65.8 ± 44.0 (n = 45)	57.5 ± 32.5 (n = 11)
1998 (n = 457)	81.3 ± 50.2 (n = 207)	71.5 ± 46.9 (n = 125)	83.4 ± 50.6 (n = 73)	86.8 ± 48.9 (n = 52)

* All values are expressed as the mean ± standard deviation ($\times 10^6$/mL). No significant difference was recognized by analysis of variance.

counts in healthy Japanese men, it seems to be relevant to examine the effect of environmental estrogens on male gonadal functions. However, no reliable study on this subject has been performed. Therefore, the current study was conducted.

In this study, no deterioration in sperm counts in healthy males over the past 20 years was found in the Sapporo area of Japan. Of course, we should make careful efforts to interpret this result. It is obvious that an epidemiological study such as the current investigation of sperm counts often contains many inherent biases, including the selection of participants, geographic region of the study, definition of normal men, specimen collection methods and measurement errors, and statistical analysis (Swan et al, 1997). Although careful efforts were made to reduce these biases in the current study, an issue in relation to the selection of volunteers still remained. This is a crucial point to assess the results in comparison to other reports.

Many studies were designed using a limited homogeneous mass; they contained biases in the selection of subjects, such as donors of sperm, university students, or patients who visited male infertility clinics. It is important that the results can be extrapolated to a current statement of sperm counts in the general population. Ideally, a community-based or population-based study would be most appropriate to reflect the general male population; however, it seems to be very difficult to perform a sophisticated epidemiological study in this field. It is not easy to collect semen samples even if participants are cooperative. Moreover, in the 2 studies, all volunteers registered themselves as a result of their interest and in response to handbills in 1998 and posters in 1975–1980. Thus, one of the problems in these studies is that subjects were self-selected volunteers.

Handelsman (1997) recently pointed out the invalidity of extrapolating similar results on sperm counts of self-selected volunteers to the general population of men. As for the method of recruiting volunteers, the materials used were different between the 2 studies; that is, handbills and posters. Moreover, the big difference between the 2 studies was whether inducement for participation was money. Volunteers were paid in 1998, but not in 1975–1980. The 1998 study may have been weighted toward younger men because they were paid for their registration. There may exist various differences in the sources of populations between the 2 studies. These differences may also have affected the results of sperm counts. Thus, we should remember these differences in recruiting volunteers when we interpret the results, and that it is difficult to extrapolate the results to the general population.

As for methodological issues, including specimen collection and measurement of sperm counts, the 2 studies were performed under generally the same conditions to reduce methodological biases. However, one possibility that may have affected the results was the period of abstinence. Because the criterion of abstinence for entry into this study was 3 days or more, the period of abstinence may not have been the same among the volunteers and between the 2 studies. Because the exact number of days of abstinence was not evaluated, this may have affected sperm counts.

The rate of subjects who had a child was approximately the same in the 2 studies; however, it was of interest that the mean sperm count was significantly higher in the 1998 study than in 1975–1980 in fertile subjects. Why the mean sperm count in fertile men increased in the current study compared with the earlier study is an unanswerable question. The hypothesis arose that more sperm were required to fertilize the ovum, although the mean sperm count did not deteriorate over the 20 years. Was there any functional impairment of sperm that caused the requirement of more sperm for fertilization? The number of subjects in this study was too small to answer this question and a further prospective study is needed.

The question of whether the results of the current study reflect those of all healthy, young Japanese men is unresolved. Differences of sperm counts among the regions where the studies were performed were recognized even in the same country (Auger et al, 1995; Bujan et al, 1996; Fisch et al, 1996). In France, a decline in sperm count has been reported in Paris during the past 20 years (Auger et al, 1995), whereas the sperm count was not changed with time in the Toulouse area (Bujan et al, 1996). The same results were found in the United States (Fisch et al, 1996). Hence, to confirm the actual condition of sperm counts of all Japanese men, a carefully designed prospective study using the same methods such as recruitment of volunteers, collection and measurement of semen, should be performed in several regions of Japan.

It is suspected that environmental endocrine disrupters can affect male gonadal development during the fetal period (Toppari et al, 1996). Therefore, accumulation of these environmental endocrine-disrupting chemicals in women could be a serious problem because of exposure of the fetus to these toxic products during pregnancy. Recent scientific and industrial development might have exponentially enhanced our chances of exposure to many endocrine-disrupting chemicals. The possibility that male reproductive function deteriorates due to frequent exposure to such endocrine-disrupters would be higher in the present and the future than in the past. Although there was no deterioration of the sperm counts over the past 20 years in the Sapporo area, it is not guaranteed that reproductive function in men will be maintained at the same level for the next 20 years. Based on these considerations, prospective monitoring of sperm counts should be carried out in the future to assess the changes in male gonadal

function, although no evidence of male gonadal dysfunction is recognized in the current situation.

In conclusion, this is the first reliable report about sperm counts in healthy, young Japanese men. No deterioration of sperm counts was recognized in the current study; however, a well-designed prospective study should be prepared and performed to resolve the worldwide question of whether a deterioration in the reproductive function of men has occurred.

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