Semen quality in Greenland

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ABSTRACT
Objectives. To quantify semen quality in the Greenlandic population. Study design. A cross-sectional study including recently proven fertile men from four regions including nine municipalities and one settlement in Greenland. Methods. The samples were analysed for sperm cell concentrations and motility using standard methods. Results. In total 201 semen samples were collected. The median sperm cell concentration of fertile men in Greenland was 53 x10⁶ sperm cells/ml, with a median sperm cell volume of 3.2 ml, a total sperm count of 185.6 x10⁶ sperm cells and a median motility of 60%. Regionally there were no statistical significant difference in sperm counts, but the sperm cell motility differed among regions, with the lowest sperm cell motility found in semen samples collected on the east coast. Overall sperm cell concentration and total sperm count in Greenland seems to be in the lower range compared to studies from Europe, USA and Japan. Conclusion. Within Greenland the sperm cell concentration appears to be similar in different regions, but sperm cell motility tends to be lower in men from the east coast. This may be related to the higher organochlorine exposure level previously demonstrated in this population.

Keywords: Sperm count, Greenland, biopersistent organochlorines, PCB, DDE

INTRODUCTION
Several studies suggest temporal shifts and geographical differences in semen quality (1-6) but the causes are not known. The possible importance of environmental exposure to chemicals has raised considerable concern (7), but most semen studies performed in the general population include a limited number of subjects or lack appropriate exposure measures (5, 8). Some of these methodological limitations are addressed in a reproductive study of Inuit and European populations with focus on exposure to the biopersistent organochlorines PCB and DDE. PCB and DDE are among the chemical contaminants found in the highest concentration in human blood samples, and especially in the Inuit populations they are found in high concentration due to bioaccumulation of these compounds in the traditional Inuit food. The largest proportion of biopersistent organochlorines in Inuit blood and tissue samples have been indicated to originate from the consumption of sea mammals (9). In this paper we report on regional differences in sperm count and motility among the Inuit population of Greenland, and compare the semen quality in Greenland to previously published studies from other countries.

MATERIALS AND METHODS
The semen study was conducted in October 2002 and in May through August 2003 in nine municipalities and one settlement located in four regions in Greenland [1] Ilulissat and Qeqertarsuaq in the Disco Bay area, 2) Sisimiut, Maniitsoq and Nuuk on the central west coast, 3) Narsaq, Qaqortoq and Nanortalik in southern Greenland and 4) Tasiilaq and Kuummiut (settlement) on the east coast]. 62% of the Greenlandic population live in the selected areas (Statistics Greenland 2003 http://www.statgreen.gl).
The inclusion criteria for this study were: 1) The spouse/partner should be or should have been pregnant within one year. 2) Both the pregnant woman and the male spouse/partner should be more than 18 years of age and 3) they should both be born in Greenland. All pregnant women, identified by the local midwife at the time of visit in the selected areas, were addressed, and if the inclusion criteria were fulfilled they and their spouse/partners were asked to participate in the fertility study, which the semen study is part of. Most of the areas were in addition visited for recruitment up to one year prior to the semen collection. Men having demonstrated fertility within one year were included in the semen study.

Semen samples were collected by masturbation at the residence. The subject was asked to abstain from sexual activities for at least two days before collecting the sample, if possible, and to note the actual abstinence time. The sample was kept close to the body to maintain a temperature close to 37°C when transporting it to the local hospital immediately after collection. Here the samples were analysed for motility and concentration according to a manual for the project based on the WHO manual for basic semen analysis (10). Using a microscope mounted with a heated stage (37°C), sperm cell motility was determined by counting the proportion of a) fast progressive sperm; b) progressive sperm; c) local motile sperm; and d) immotile sperm on 100 sperm cells within each of two fresh drops of semen, placed on a preheated (37°C) clean glass slide, and covered with a coverslip. On all samples the sperm cell motility was analysed within one hour after ejaculation. The sperm cell concentration was determined on diluted semen samples (1:10 or 1:20) using an Improved Neubauer Hemacytometer. All semen samples were analysed by one researcher who had been trained for human semen analysis.

The data from Greenland were compared to recently published studies of semen quality of men with pregnant wives in Europe [Denmark, France, Scotland and Finland (5)], United States [Missouri, New York, Minnesota and California (6)] and Japan (11).

**Statistical analyses.**
The distribution of semen characteristics across the four regions of Greenland was examined by analysis of variance. To obtain homogeneity of variance and normality, the total sperm count, volume and period of abstinence time were transformed by the natural logarithm. Since the sperm cell concentration, volume and total sperm count were associated with abstinence time, the results given in the statistical analysis are adjusted for abstinence time for these outcomes, whereas no adjustment was done in motility analysis. Inclusion of the following covariates: recent fever; reported spillage of the semen sample; infections, vaccinations or operations; or medicine intake up till 6 months prior to the sample was taken, did not significantly change the conclusions, and these covariates were therefore not included in the model. Multiple comparisons among regions were done with the LSD method. Comparisons of proportions among regions were performed by Fisher’s exact test. All statistical analyses were performed using SPSS release 10.0.5 (SPSS Inc. Chicago, USA).

**RESULTS**
Of the 257 eligible men 201 provided a semen sample, giving a participation rate of 78%. Overall in Greenland, the sperm cell concentration ranged from 0.6 to 374 x 10⁶/ml, with a mean of 72.0 and median of 53 x 10⁶/ml (table I). The total sperm count ranged from 2.4 to 1496 x 10⁶ (mean 247.2 x 10⁶; median 185.6 x 10⁶), the volume ranged from 0.7 to 9.7 ml (mean 3.5 ml; median 3.2 ml) and the proportion of motile sperm cells (grade a+b) ranged from 1 to 87% (mean 55.0 %; median 60 %). The sperm cell concentration, total count, volume and motility by region are presented in Table I. The sperm cell concentrations and total sperm counts were not statistically significantly different among regions, but the semen volume differed among regions, with significantly lower sperm cell volume in south and east Greenland compared to the central west coast region (p=0.01 and p=0.05, respectively), and the sperm cell volume in south Greenland also appeared to be lower compared to the Disco Bay region (p=0.02).
Also sperm cell motility differed among regions, with the lowest sperm cell motility found in samples from the east coast. The sperm cell motility in the east coast samples was significantly different from the samples from the Disco Bay area (p=0.01) and south Greenland (p=0.01). Also the proportion of persons with low sperm cell motility which is defined by WHO (10) as less than 50% a+b motile sperm, was significantly lower in samples from the east coast regions compared to the Disco Bay area (p=0.02) and south Greenland (p=0.01) (table II). The proportion of men with low sperm counts (< 20 x 10^6 sperm cells/ml) was not statistically significant different among regions.

The sperm cell concentration, volume and total sperm counts in recent comparable studies are presented in table III.

**DISCUSSION**

The study presented here is the first study of Inuit semen quality to date. There appear to be no major differences in sperm cell concentration or total sperm count among the investigated regions in Greenland, but the semen volume differed among the studied regions. Semen volume has been demonstrated to have a weaker predictive value for human fertility than sperm cell concentration (12), and therefore we do not believe that this has

**Table I.** Sperm cell concentration, total sperm count, volume and motility by region and in total in Greenland.

| Region (sample size) | Concentration x 10^6/ml Mean (SE) | Median | Total sperm count x 10^6 Mean (SE) | Median | Volume ml Mean (SE) | Median | Motility grade a+b % Mean (SE) | Median |
|----------------------|-----------------------------------|--------|-----------------------------------|--------|---------------------|--------|---------------------------|--------|
| Disco Bay (53)       | 76.1 (9.1)                        | 62.5   | 295.2 (41.0)                      | 215.2  | 3.7 (0.2)           | 3.4    | 58.3 (2.4)                | 63.0   |
| Central west coast (88) | 71.6 (6.6)                     | 49.0   | 256.3 (25.1)                      | 182.5  | 3.8 (0.2)           | 3.4    | 53.8 (1.9)                | 56.0   |
| South Greenland (35) | 63.0 (7.6)                        | 52.1   | 166.3 (24.2)                      | 109.6  | 2.9 (0.2)           | 2.5    | 59.1 (3.1)                | 64.0   |
| East Greenland (25)  | 77.5 (13.3)                       | 63.5   | 227.3 (52.1)                      | 146.7  | 2.9 (0.3)           | 2.8    | 46.8 (4.4)                | 43.0   |
| Greenland total (201)| 72.0 (4.3)                        | 53.0   | 247.2 (17.4)                      | 185.6  | 3.5 (0.1)           | 3.2    | 55.0 (1.3)                | 60.0   |

**Table II.** Percentage of the population with low sperm quality based on WHO standards.

| Region (sample size) | Low sperm count < 20 x 10^6 sperm cells/ml | Low sperm cell motility < 50% a+b motile sperm |
|----------------------|---------------------------------------------|-----------------------------------------------|
| Disco Bay (53)       | 11.3                                        | 26.9                                          |
| Central west coast (88) | 13.6                                        | 36.4                                          |
| South Greenland (35) | 17.1                                        | 20.0                                          |
| East Greenland (25)  | 16.0                                        | 56.0                                          |
| Greenland total (201)| 13.9                                        | 33.5                                          |

**Table III.** Recent studies of semen quality of men with pregnant wives in Europe (5), USA (6), Japan (11) and Greenland (this study).

| Region/Country/State (n) | Participation rate % | Median Concentration x 10^6/ml | Mean Total sperm count x 10^6 | Mean Volume ml |
|--------------------------|----------------------|--------------------------------|-------------------------------|----------------|
| Europe                   |                      |                                |                               |                |
| Denmark (349)            | 43                   | 61                             | 276                           | 3.8            |
| France (207)             | 15                   | 74                             | 385                           | 4.2            |
| Scotland (251)           | 77                   | 82                             | 412                           | 4.1            |
| Finland (275)            | 51                   | 53.5                           | 229*                          | 3.9            |
| USA                      |                      |                                |                               |                |
| Missouri (176)           | 40                   | 88.5                           | 340*                          | 3.3            |
| New York (38)            | 54                   | 81.8                           | 384*                          | 3.9            |
| California (124)         | 37                   | 64.8                           | 291*                          | 3.6            |
| Japan                    | 22                   | 82.3                           | 345*                          | 3.2            |
| Greenland (201)          | 78                   | 53.0                           | 247                           | 3.5            |

* Estimated from mean concentration and mean volume
any major significance on the fertility among the regions. However, another significant predictor of male fertility, sperm cell motility, (13, 14) tended to differ among regions, with the lowest motility found in the semen samples collected on the east coast. It is interesting to note that previous data indicate that the highest concentration of PCBs and DDE are found in serum samples from people from the east coast compared to other regions in Greenland (15). This suggests that sperm cell motility may be related to organochlorine exposure. This is further supported by recent findings. In a study from Sweden a weak but significant correlation between sperm cell motility and PCB-153 was found among men from the general population (16). Sperm cell motility has also been demonstrated to be affected in case control studies of highly exposed populations (17, 18). Analysis of PCB and DDE are included in future studies on blood samples from the men involved in the semen study, and after these analyses have been completed we will know more about the potential association between exposure to biopersistent organochlorines and sperm quality in the Greenlandic population.

Sperm cell concentration, which has been indicated to be of high predictive value to male fertility (12), seems to be in the low end in Greenland compared to other studies (table III). Higher median sperm counts were found especially in Finland (77x10^6/ml), Japan (82.3x10^6/ml), New York (88.5x10^6/ml) and Minnesota (81.8 x10^6/ml), whereas only slightly higher sperm cell concentrations were found in Denmark (61 x10^6/ml) and Missouri (53.5 x10^6/ml). None of the studied populations had lower sperm cell concentrations than the Greenlandic population, and since the European and American study showed significant differences among regions (5, 6) it can be suggested that the Greenlandic sperm cell concentration is lower compared to at least some of the other studied populations. The mean semen volume in the fertile men from Greenland was in the same range as in other international studies, reporting a mean semen volume in the range from 3.2 to 4.2 ml. Therefore, also the total sperm count was in the lower end compared to other international studies, reporting a mean range from 229 to 412 x10^6 sperm cells (Table III). However, strong inferences should not be made from comparisons of crude semen data across countries. Besides differences in recruitment and participation rates it is not possible to control for confounding factors such as period of abstinence.

The assessment of sperm cell motility has not been as standardized as the other reported measures of sperm quality, and therefore the data from the Greenlandic study cannot be directly compared to the other populations.

A high participation rate in semen studies is a key factor to avoid bias. In the present study the participation rate of 78% was among the highest reported for semen studies, with previous studies reporting a participation rate in the range of 15-54% (table III). In order to try to get a high participation rate in the present study, all potential participants were individually explained about the project in their native language by a medical doctor directly involved in the project. Furthermore, the Greenlandic media have several times reported on the exposure of Inuits to biopersistent organochlorines. We believe that the personal information about the project together with the high awareness in the Greenlandic population about the possible adverse effects on human health of biopersistent organochlorines are some of the main factors that increased the willingness to participate in this study.

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