

# Regional Differences and Temporal Trends in Semen Quality

**Niels Jørgensen**  
Rigshospitalet, Denmark

Thank you for your introduction and also I would like to thank the organizers for inviting me to come to Japan and speak of temporal trends and regional differences in semen quality. It is a large topic that could take many hours to cover, but I will try to restrict myself and keep within the time limit I have been given.

The recent discussion of a change in male reproductive health started with the publication in 1992 in the British Medical Journal by Elisabeth Carlsen and co-workers from our group in Copenhagen. They indicated that there had been a decline of around 50% in mean sperm count over a 50-year period.

They based the analysis on 61 previously published data publications. Of course it raised a lot of debate. Some argued that maybe the right statistical methods were not used, maybe it was not be right to pool data from all over the world into one analysis, and also several people tried to reanalyze the data included in the Carlsen paper.

In the year 2000, in Environmental Health Perspectives, Shanna Swan did not only make a reanalysis, but she also included extra publications, altogether 101, and controlled for the period of abstinence, reason for sampling, and so on, and divided the groups into men coming from Europe, men from North America, and then from other parts of the world and confirmed that there had been a decline at least in Northern Europe and in North America. They were not able to conclude anything from the non-western countries from where the data were very limited.

Following these meta-analyses, several groups of people went back and looked into their own archives and investigated the data they had themselves. Some showed no decrease over time; it was from the United States, Fish and Paulsen who could not find any change over time in their materials.

Also from Finland in the northern part of Europe, Vierula and co-workers could not detect any change over time based on data collected over a 28-years period. They reported that the Finnish men had a high and unchanged semen quality. In contrast other studies from Europe were able to show a decrease. Auger and co-workers from France had for many years investigated potential semen donor candidates and showed that there among these men had been 2.8% decrease in sperm concentration per year over a 20-year period.

Stewart Irvine in Scotland did more or less the same and showed there had been a decrease, but what he also did was to group the men according to their year of birth, and it appeared that men born recently had a lower sperm concentration than men born earlier and that was irrespective of the men's age and the time of the delivery of the semen sample.

As I mentioned, Fish in the United States was not able to show any change over time. But they pointed out large differences. Men in New York had an apparently very high sperm concentration, whereas men from Los Angeles only had half the sperm concentration.

What has happened in the past is very difficult to say, and I think that most will agree that we will never really learn from these retrospective studies because they were designed for other purposes and not to investigate whether there had been a temporal change over time. But if we should conclude something from these studies, it is that they indicate there is a geographical difference between countries. There are some studies that were not able to show a decrease in sperm concentration over time, but overall there may have been a decreasing trend. This decreasing trend may be a birth cohort effect indicating that something may have happened prenatally or perinatally.

What we then did was to set up 2 international semen studies. Of course, they will not tell us what has happened, but we hope they can tell us what happens from now on.

The first study we designed was a study of fertile men. We called it the study of partners of pregnant

women, because we included fertile men while their female partners were pregnant. The pregnancy should be achieved by normal sexual relationship and not by some sort of fertility treatment.

Of course, these men are not representative of men from the general population because they are selected due to their fertility. What we really want is to investigate men from the general population, which I will also come back to.

These two studies have taken place in the northern part of Europe. Currently, there are also ongoing 2 studies here in Japan under supervision by Prof. Iwamoto, but I will not go into that because he will tell about these studies himself.

Also in the United States, a study of fertile men is ongoing and the first publications have already come out. These studies are under the supervision by Shanna Swan.

In both of the two European studies, the men had to answer a very comprehensive questionnaire. For the study of the fertile men, also the women had to answer a questionnaire. The men had to undergo a physical examination where we mainly focused on the andrological phenotype. The men had to have a blood sample drawn for hormonal assessment. And, of course, they had to deliver a semen sample for semen analysis.

All results have then been entered into a centralized database we have in Copenhagen. We have a centralized database both to be sure that the data entering procedure is done properly but also having all the data together makes it much easier for comparisons, afterwards.

Of course, we used the same protocol. That is not a surprise for anyone, I will guess. But also assessment of semen quality of a semen sample is not so simple as it may seem to be. We found it necessary to specify the semen analysis procedures more than what is said in this WHO manual.

Also we required that the laboratories participated in an external quality control study so we were able to control for the inter-laboratory variation. Morphology smears were assessed in one laboratory only, and all the hormones were analyzed in our laboratory in Copenhagen, also again to reduce the inter-laboratory variation.

The first study was the study of fertile men. It took place in Denmark, Finland, Scotland and France and has been published already. We included altogether almost 1,100 men.

The study took place covering at least a 12-month period and that was because we wanted to be able to take into account a possible seasonal variation. In this study it appeared there was a variation, and the same variation in all 4 centers: Turku, Finland; Copenhagen, Denmark; Paris, France; and Edinburgh, Scotland. In all centers it seems as if sperm concentration was higher during winter than during summer. In summertime, it was only around 70% of the winter level.

Also, we showed that there was a difference between men from Finland and men from Denmark. Finnish men had the best sperm count, and Danish men had the lowest. The men from Paris, France were at almost the same level as the Danish men, whereas the men from Scotland were closer to the Finnish level than to the Danish.

But the results of the study also emphasized the importance of taken this seasonal variation into account, because if we - in this study - had all the Finnish men examined during summertime and all the Danish men during wintertime we could have reached a conclusion that there was no real difference between Denmark and Finland, or perhaps even that the Danish men had the best sperm counts which overall was not the case. It is the Finnish men who have the best sperm concentrations.

If we then turn to total sperm counts which more or less reflects the same because there is no difference in volume between the centers, it is the same pattern, with Finnish men having the best, the Danes the poorest count, and men from France and Scotland in between.

The count is not everything; there is also motility. For motility we were not able to show any seasonal variation in this cross sectional study; also we were not able to show any meaningful difference between the

centers. In all four centres it was between 60% and up to 65%. So from a biological point of view there was no real difference.

In this study, all the morphology smears were assessed in Paris, France in Pierre Jouannet's laboratory and where they used the David criteria. There were no seasonal variation and no difference in percentages of normal forms. So in this study of fertile men we were able to show a difference in total sperm count and concentration but not in motility and morphology. But nevertheless, even though it was a group of fertile men, we were able to show a difference.

Then, the first study of young men from the general population was undertaken in Copenhagen. Here I need to tell how we include young men from the general population.

In Denmark all young men have to attend a compulsory medical examination when they are 18 years old to declare whether they are fit or unfit for military service. Only those who are severely handicapped are excused; that means very few in reality. So, all young men have to show up there, and we included men to our study when they showed up for this examination irrespective of whether they were declared fit or unfit for service. The first study was undertaken from autumn 1996 to spring 1998 and we included 708 men.

It appeared that these young men that were 19 years of age when we investigated them had a very low sperm concentration, only 42 million/ml, which is less than half of the fertile Danish men. This study was only undertaken in the winter season.

Of course, it is not a surprise that men from the general population will have a lower sperm concentration than fertile men, because these young men do not know anything about their own fertility and some of them will later show up to be infertile or subfertile. Nevertheless, this difference between these young men and the fertile men was very surprising for us.

The countries like Finland, Norway and Estonia have the same military drafting system as we have in Denmark. Therefore, we included altogether almost 1,000 men from these four countries. The men were born in 1979, 1980, or 1981.

Again, like the study of the fertile men, the Finnish men had the highest sperm concentration and the Danish men the lowest concentration. But you will also note that these Finnish men had a lower sperm concentration than the fertile Finnish men, so it is actually a rather low level also for Finnish men, but still they are at a higher level than the Danish men.

Together with the Finnish men are the Estonian men with a high concentration. Finland and Estonia are situated in the eastern part of the Nordic-Baltic area. The Norwegian men have the same low sperm concentration as the Danish men and both countries are from the western part of the Nordic-Baltic area.

If we look at the total sperm counts, again the same pattern: highest counts in the eastern part, lowest in the western part of this area. For motility, no meaningful difference.

Morphology in this study was assessed in Turku in Finland according to strict criteria. All samples were assessed by one person, and here it came up that there was a difference in percentage of normal forms, again the Finnish men and the Estonian men had the highest percentages and the Norwegian and Danish men the lowest; so there is a regional difference.

What are all the explanations for this? I cannot tell you, but the available literature indicates that environmental factors have caused the adverse trends seen in male reproductive health. We have currently no solid explanation what may cause the regional differences I have described from these 2 studies. But we have a lot of information from questionnaires, from blood samples, and from the physical examinations that we still have to analyze completely.

Hopefully, we can get some ideas of what have caused the differences, but such analysis will of course, not prove any causal relationship between exposure and following semen quality, but they may indicate some causes which then can be evaluated in other studies designed for that.

So just to repeat: for the fertile men the lowest counts in Denmark, highest in Finland. For the young

men, low counts in Denmark and Norway, higher in Finland and in Estonia. Secondly, a Lithuanian group have used our protocol and participated in the quality control study completely and have just published in the International Journal of Andrology that Lithuanian men have a high semen quality comparable to the Finns and Estonian men.

Also, recently there has been a publication in Human Reproduction of a semen study taking place in the southern part of Sweden. That group reported a relatively high concentration for these Swedish men closer to the Finnish level than to the Danish level. The group used our protocol, but unfortunately they did not participate in our quality control study, so we do not know exactly whether we can rely on that figure or some of the differences may be caused by a difference between assessment levels in laboratories.

I should say something about time trends in Denmark. As I said, it is very difficult because there have not been any really good studies for that yet. But in the 1980s and 1990s Jens-Peter Bonde looked at workplace exposures and have 10 publications from that, and if we pool data from these 10 studies and group men according to the year of birth, it appears that, men born earlier had higher counts than men born recently.

Then, what about here? If we then include the young men we investigated from the first Danish cohort, it appears as if there is a line that the youngest men, the men born most recently, have the lowest sperm concentration. But of course these groups are not completely comparable: this is one kind of group from the work exposure group and this is the young men.

Semen quality is not everything when it comes to male reproductive health. Danish men have a leading record, unfortunately, too with the highest incidence of testicular germ cell tumors, followed very closely by the Norwegian men. That means that men from countries with the lowest sperm concentration also have the highest incidence levels of testicular cancer.

In contrast, men in Finland have a very low incidence of testicular germ cell cancers, and at the same time Finnish men seem to have the best sperm quality.

The incidence has been increasing steadily in Denmark and in Norway over many years. It may also be increasing in Finland but at a much, much lower level than seen in Denmark. Another aspect that I will just not mention too much is that there may be a high incidence of cryptorchidism among Danish boys and lower in Finland; again, poor situation in Denmark, better in Finland.

Recently, Prof. Skakkebaek from our group in Copenhagen has suggested what he calls a Testicular Dysgenesis Syndrome. That is based on the idea or the fact that testicular cancer is actually a fetal disease that just manifests itself later in life, and also perhaps impaired spermatogenesis may be a result of some prenatal factors acting there as well. Thus, these two diseases may share prenatal causes together with the undescended testes and the hypospadias. I think I will not go further into that because it will be much clearer when Jorma Toppari has given his talk on the cryptorchidism and hypospadias data.

Of course, I have not done all of the work, I am just one of the persons involved in the studies. There have been collaborators from Estonia, Finland, France, Norway and Scotland. But I will mention Prof. Skakkebaek from our group in Copenhagen who is the leading force behind all of the studies that I have reported here. Thank you for your attention.

## Q&A

Iwamoto: I would like to get questions or comments now.

Q: That was an interesting presentation, and I was wondering, I was at a presentation last week where Dr. Fish suggested that some of the incidences of testicular cancer were associated with a higher age of conception for the parents, and I was wondering if in the data that you presented today, if that factor had been accounted for or considered.

Jørgensen: In the data I presented, no, we are not taking that into account. But we have the information in our questionnaire, and this is one thing that we are going to look at, the parents' age.

One inclusion criteria was that the fertile men should be at least 18 years old and not more than 45 years old. In median they were almost 32 years old in Denmark. These data I presented were adjusted to men 30 years old, for the fertile men. The young men they are much younger, they are 18, 19 or 20 years old, in median around 19 years of age.

Q: Thank you.

Jørgensen: You are welcome.

Matsuzaki: I think I might have misunderstood something, so I'd like to ask you a question. You showed us the normal morphology percentage. The graph shows that 50% for a 30-year-old male would be normal and 10% or less for a 19-year-old male. Is this all right?

Jørgensen: In this study of the fertile men, all of the morphology samples were assessed in Paris. In Paris they used what is called the David criteria which is very close to the old fashioned WHO criteria and that made the high percentages here.

We have also reevaluated all the morphology slides from the study of fertile men using the strict criteria that we are currently using for the young men study, and when we evaluated the slide of the fertile men according to strict criteria then the frequency of normal forms turn down to around 15%. I should have shown it, but I did not.

Matsuzaki : I understand. Thank you.

Jørgensen: You are welcome.

Q: When I saw the other data that shows a high incidence of testicular cancer in Denmark, I thought it was odd. You however connected this clearly with sperm count in your presentation today, so I think the presentation will have a huge impact.

What I'd like to know however is if such deterioration of seminal fluid occurs, I think it would be largely connected with the country's diet. Have you done any research in this area. If so could you tell us about it?

The question was whether diets, for example in Finland and Denmark, could explain the difference for sperm counts and testicular cancer.

Jørgensen: The short answer would be I do not know. This is what we hope to elucidate when we look into all this questionnaire information. We have a lot of information on diet.

Also we tried to go back and get some information of prenatal exposure because we asked the men to go back and ask their mothers to fill in a certain part of the questionnaire. Currently, I cannot tell that there are any striking differences between the food we eat in Finland and Denmark. This is what we are going to look into.