

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Human Semen Quality in the New Millennium: A prospective cross sectional population-based study of 4,867 men
AUTHORS	Niels Jørgensen, Ulla Nordström Joensen, Tina Kold Jensen, Martin Blomberg Jensen, Kristian Almstrup, Inge Ahlmann Olsen, Anders Juul, Anna-Maria Andersson, Elisabeth Carlsen, Jørgen Holm Petersen, Jorma Toppari and Niels E. Skakkebæk

VERSION 1 - REVIEW

REVIEWER	<p>Professor Richard Sharpe Research Group Leader MRC Centre for Reproductive Health The Queen's Medical Research Institute University of Edinburgh, UK</p> <p>Conflicts of interest I have been a co-holder of EU grants with some of the authors and have co-authored three review/hypothesis manuscripts with the senior author. However, I have never been involved in any aspect of the semen analysis studies in young men, including those currently reported.</p>
REVIEW RETURNED	18/02/2012

RESULTS & CONCLUSIONS	As I point out in my comments below, I think the main (important) messages from this study are not as clear as they should be, so some rewriting of the discussion is warranted
GENERAL COMMENTS	<p>The issue of falling sperm counts has been with us for 20 or more years and remains both controversial and unresolvable (we cannot go back in time and check the data). We can therefore only deal with the present and recent past and relate this to this issue. The Copenhagen group and collaborators have been virtually the only group in the world to proactively adopt this painstaking approach, all others have mainly continued to argue their positions (vis a vis 'falling sperm counts') without collecting new data.</p> <p>In essence the present study deals with the results of the 'Copenhagen approach' over the past 15 years. It is founded in the normal population (of young men) and has used standardized recruitment and analysis criteria throughout. It shows two important facts. First, that throughout the 15 years, average sperm counts in young Danish men have remained consistently low when compared with historical data from the literature, and with Danish data for fertile men (previously published) and, most remarkably, with data from the 1940s for Danish men in infertile couples; considering that the latter men are established to have lower average sperm counts as a group that do men from the normal population, this comparison provides yet another strand of evidence that supports the notion that sperm counts were historically higher than at present (using the same basic counting method/slides). Second, the 15 year analysis shows that</p>

	<p>over that period ~20% of Danish young men have had a sperm count low enough to impair their fertility; the authors have also shown that this is unchanged when monitored over a number of years for individuals (ie their sperm count when first measured is a reflection of what it is likely to be throughout adulthood). There is also a third element to emerge from the 15 year study and that is evidence for a small upward trend in sperm count, although the data are not especially convincing that this represents a genuine trend.</p> <p>Although it may be argued that this manuscript represents nothing more than just semen quality analysis, which is hardly new, in fact this study represents a unique and unmatched database that carries with it several important messages. The studies cannot be faulted technically or in design or execution. It arguably provides the most sensitive barometer available of the reproductive health status of the next generation of men in Denmark (and probably Europe as a whole). For all its simplicity, it is therefore invaluable.</p> <p>I have a number of comments to be addressed:</p> <ol style="list-style-type: none"> 1. The authors emphasize the consistently poor sperm morphology in men across the study period, but to an extent I find this overdone. It (unintentionally I imagine) implies that this may have changed for the worse with time, whereas there is no very convincing evidence that this is the case. It may serve as a reminder to readers about the generally poor quality of human sperm, but this may be an evolutionary relic (lack of selection pressure on sperm quality in monogamous, life-bonding humans, in contrast to the opposite in most animals). I agree that it is worth emphasizing in the context that men are poorly placed to also suffer erosion of sperm counts, but I would suggest that the authors reconsider how this aspect is presented. 2. The authors have reported and interpreted their findings in a commendably measured (even restrained) way, but this has been overdone, to the extent that the really important meaning and messages are sort of drowned in the 'mediocrity' of semen analysis per se. What is desperately needed is a more focused 'pulling together' of the presented data in the discussion in relation to its implications for (i) 'fallen sperm counts', (ii) present status of male reproductive health, and (iii) implications of the latter for couple fertility in the present environment. All of this is discussed or mentioned in passing, but not in as cohesive, constructive way. The data certainly warrants this. This study is much, much more than just a huge amount of semen analysis down the years, and therefore needs to be sold as such. It has important messages for researchers, health regulators and, most importantly, for individuals/couples, but these do not emerge as the take home messages, and they should. 3. The MS is very clearly written, but I found one potential ambiguity (Page 9, lines 11-16) – it is not clear what data are being referred to in the 'cut-offs' in fertile men, so slight rewording would help.
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REVIEWER	<p>Bernard JÉGOU Director of Research Inserm Rennes, FRANCE</p> <p>I, Bernard Jégou, declares that no competing interests exist.</p>
REVIEW RETURNED	03/05/2012

GENERAL COMMENTS	<p>OPEN BMJ</p> <p>Since the beginning of the 90's there has been a fierce debate on whether or not a deterioration of the semen quality of men had occurred. The debate was initially ignited by the now famous meta-analysis by Carlsen et al (1992, citations >1200), elaborated and performed by the same laboratory as the one which has produced the present study. In this context, one of the key elements of the scientific controversy was that all the studies performed in the domain were retrospective studies with the inherent bias attached to them.</p> <p>Therefore, the need for quality controlled prospective studies has been recurrently put forward in many instances and at different levels. However, to the best of our knowledge over the last 20 years only three studies have actually been conducted on a prospective basis, all in the Northern Europ (Finland, Sweden and Denmark). Clearly, the present study is the most important investigation yet performed.</p> <p>It presents a number of important assets:</p> <ul style="list-style-type: none"> - it is a well designed and controlled cross-sectional study including a large number of men (> 4 000) from the general Danish population and covering a period of 15 years (1996-2012); - it has been performed under strict quality control procedures; - it concerns a country –Denmark- which is known to present some of the highest incidence rates of cryptorchidism and testicular cancer in the developed world. <p>Therefore, there was a high pertinence at also investigating the evolution of the semen quality since, the different parameters of the male reproductive health are possibly interconnected;</p> <ul style="list-style-type: none"> - in addition to this prospective study which represents the core of the paper, the manuscript also compares the data obtained by the authors to the data of a smaller follow up study of men who recently became partner, and of historical data (doctoral thesis published in 1944). <p>According to the data presented in this manuscript an increasing trend in sperm concentration and total sperm count has occurred in Copenhagen over the 15 years covered by the study. However, the authors confirmed and reinforced the observations that only one in four men in Copenhagen had optimal semen quality, with no indication of improvement with time. The study also suggests that Danish men of the XXIst century have lower sperm counts that the men of the historical cohort of reference (Hammen cohort).</p> <p>The paper is well designed and very well written. Considering the exceptional experimental effort realized, the quality of the critical discussion of the manuscript, and the various aspects of the results generate from a fertility perspective, the reviewer strongly recommends its publication.</p> <p>Comment:</p> <p>All the papers (the few papers) corresponding to prospective studies on semen quality should be discussed including the Axelsson et al paper in Human Reprod (26, 2011) which has not been quoted in the manuscript.</p>
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REVIEWER	<p>Prof. Dr. Maria Blettner Institute of Medical Biometrics, Epidemiology and Informatics (IMBEI) Obere Zahlbacher Str. 69 55131 Mainz Germany</p>
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	I have no competing interests.
REVIEW RETURNED	09/05/2012

THE STUDY	Please, give details regarding the achieved response rate in the section "Materials and Methods" and the discussion.
GENERAL COMMENTS	Tests for solution bias (page 9, line 55-59): Give more details on the number of subjects with blood samples. Are "hormonal levels" an indicator for semen quality?

VERSION 1 – AUTHOR RESPONSE

Reviewer, Richard Sharpe

Reviewer, Richard Sharpe: As I point out in my comments below, I think the main (important) messages from this study are not as clear as they should be, so some rewriting of the discussion is warranted.

The issue of falling sperm counts has been with us for 20 or more years and remains both controversial and unresolvable (we cannot go back in time and check the data). We can therefore only deal with the present and recent past and relate this to this issue. The Copenhagen group and collaborators have been virtually the only group in the world to proactively adopt this painstaking approach, all others have mainly continued to argue their positions (vis a vis 'falling sperm counts') without collecting new data.

In essence the present study deals with the results of the 'Copenhagen approach' over the past 15 years. It is founded in the normal population (of young men) and has used standardized recruitment and analysis criteria throughout. It shows two important facts. First, that throughout the 15 years, average sperm counts in young Danish men have remained consistently low when compared with historical data from the literature, and with Danish data for fertile men (previously published) and, most remarkably, with data from the 1940s for Danish men in infertile couples; considering that the latter men are established to have lower average sperm counts as a group than men from the normal population, this comparison provides yet another strand of evidence that supports the notion that sperm counts were historically higher than at present (using the same basic counting method/slides). Second, the 15 year analysis shows that over that period ~20% of Danish young men have had a sperm count low enough to impair their fertility; the authors have also shown that this is unchanged when monitored over a number of years for individuals (ie their sperm count when first measured is a reflection of what it is likely to be throughout adulthood). There is also a third element to emerge from the 15 year study and that is evidence for a small upward trend in sperm count, although the data are not especially convincing that this represents a genuine trend.

Although it may be argued that this manuscript represents nothing more than just semen quality analysis, which is hardly new, in fact this study represents a unique and unmatched data-base that carries with it several important messages. The studies cannot be faulted technically or in design or execution. It arguably provides the most sensitive barometer available of the reproductive health status of the next generation of men in Denmark (and probably Europe as a whole). For all its simplicity, it is therefore invaluable.

Authors reply: We agree with the comments, and have rewritten the discussion as described specifically below.

Reviewer, Richard Sharpe: The authors emphasize the consistently poor sperm morphology in men across the study period, but to an extent I find this overdone. It (unintentionally I imagine) implies that this may have changed for the worse with time, whereas there is no very convincing evidence that this is the case. It may serve as a reminder to readers about the generally poor quality of human sperm, but this may be an evolutionary relic (lack of selection pressure on sperm quality in monogamous, life-bonding humans, in contrast to the opposite in most animals). I agree that it is

worth emphasizing in the context that men are poorly placed to also suffer erosion of sperm counts, but I would suggest that the authors reconsider how this aspect is presented.

Authors reply: We have tried to strengthen the perspectives of our findings. In the abstract, we have indicated the number of men that might experience fertility problems in the future. In the discussion we have added a section focussing on the potential trend in sperm morphology vs. the suggestion that low numbers of normal spermatozoa is an evolutionary relic as suggested by the reviewer.

Reviewer, Richard Sharpe: The authors have reported and interpreted their findings in a commendably measured (even restrained) way, but this has been overdone, to the extent that the really important meaning and messages are sort of drowned in the 'mediocrity' of semen analysis per se. What is desperately needed is a more focused 'pulling together' of the presented data in the discussion in relation to its implications for (i) 'fallen sperm counts', (ii) present status of male reproductive health, and (iii) implications of the latter for couple fertility in the present environment. All of this is discussed or mentioned in passing, but not in as cohesive, constructive way. The data certainly warrants this. This study is much, much more than just a huge amount of semen analysis down the years, and therefore needs to be sold as such. It has important messages for researchers, health regulators and, most importantly, for individuals/couples, but these do not emerge as the take home messages, and they should.

Authors reply: We agree that it is important to show the important messages our results provide. At the same time we find it important to focus on technical aspects to convince readers that our results are not the result of a poor study design or non-optimal laboratory techniques, and also we should highlight the potential weaknesses. To balance these aspects we have stated that our results support the previous suggestions of an adverse temporal trend in semen quality, that more than one third of the men had impaired quality to a degree that is might impair fertility for the individual and fertility rates in the population.

Reviewer, Richard Sharpe: The MS is very clearly written, but I found one potential ambiguity (Page 9, lines 11-16) – it is not clear what data are being referred to in the 'cut-offs' in fertile men, so slight rewording would help.

Authors reply: We have rewritten this section to state that the provided cut-offs for the fertile men refers sperm concentration of 15 mill/mL and normal forms of 5%.

Reviewer, Bernard Jégou

Since the beginning of the 90's there has been a fierce debate on whether or not a deterioration of the semen quality of men had occurred. The debate was initially ignited by the now famous meta-analysis by Carlsen et al (1992, citations >1200), elaborated and performed by the same laboratory as the one which has produced the present study. In this context, one of the key elements of the scientific controversy was that all the studies performed in the domain were retrospective studies with the inherent bias attached to them. Therefore, the need for quality controlled prospective studies has been recurrently put forward in many instances and at different levels. However, to the best of our knowledge over the last 20 years only three studies have actually been conducted on a prospective basis, all in the Northern Europe (Finland, Sweden and Denmark). Clearly, the present study is the most important investigation yet performed.

It presents a number of important assets:

- it is a well designed and controlled cross-sectional study including a large number of men (> 4 000) from the general Danish population and covering a period of 15 years (1996-2012);
- it has been performed under strict quality control procedures;
- it concerns a country –Denmark- which is known to present some of the highest incidence rates of cryptorchidism and testicular cancer in the developed world. Therefore, there was a high pertinence at also investigating the evolution of the semen quality since, the different parameters of the male

reproductive health are possibly interconnected;

- in addition to this prospective study which represents the core of the paper, the manuscript also compares the data obtained by the authors to the data of a smaller follow up study of men who recently became partner, and of historical data (doctoral thesis published in 1944).

According to the data presented in this manuscript an increasing trend in sperm concentration and total sperm count has occurred in Copenhagen over the 15 years covered by the study. However, the authors confirmed and reinforced the observations that only one in four men in Copenhagen had optimal semen quality, with no indication of improvement with time. The study also suggests that Danish men of the XXIst century have lower sperm counts than the men of the historical cohort of reference (Hammen cohort).

The paper is well designed and very well written. Considering the exceptional experimental effort realized, the quality of the critical discussion of the manuscript, and the various aspects of the results generated from a fertility perspective, the reviewer strongly recommends its publication.

Reviewer, Bernard Jégou: All the papers (the few papers) corresponding to prospective studies on semen quality should be discussed including the Axelsson et al paper in Human Reprod (26, 2011) which has not been quoted in the manuscript.

Authors reply: We have added the Axelsson et al paper to the list of publications summarising results from other European countries.

Reviewer, Maria Blettner: Please, give details regarding the achieved response rate in the section "Materials and Methods" and the discussion.

Authors reply: Participation rates have been added in the Materials and Methods section.

Reviewer, Maria Blettner: Tests for selection bias (page 9, line 55-59): Give more details on the number of subjects with blood samples. Are "hormonal levels" an indicator for semen quality?

Authors reply: The number of men and participation rates together with the interpretation of the hormonal levels have previously been published (reference 14). Thus, in the discussion section we have restricted the arguments to provide the number (N=195), participation rate (79%) and information that their reproductive hormone levels including the spermatogenesis markers follicle-stimulating hormone and inhibin-b were very similar to those of the participants.