Semen quality and sex hormones among organic and traditional Danish farmers

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Abstract

***Objectives*—To confirm or refute the hypothesis that organic farmers have higher sperm concentrations than traditional farmers.**

***Methods*—Traditional and organic farmers were selected randomly from central registers, and 171 traditional farmers and 85 organic farmers delivered one semen sample before the start of the spraying season. The participation rate was 28.8% among traditional farmers and 42.9% among organic farmers.**

***Results*—The median sperm concentration for traditional and organic farmers was 58 million/ml and 64 million/ml, respectively. After adjustment for several confounders, sperm concentration, total count, proportion of non-vital spermatozoa, sperm chromatin structure, and motility variables did not differ significantly between the two groups. The traditional farmers had a significantly lower proportion of normal spermatozoa, but this result was not confirmed in a second sample. Organic farmers had slightly higher inhibin B concentration and testosterone/sex hormone binding globulin ratio.**

***Conclusion*—Despite slight differences in concentrations of reproductive hormones, no significant differences in conventional measures of semen quality were found between organic and traditional farmers.**

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In a study among members of the Danish Organic Farmers' Association, participants in a weekend seminar had a significantly higher sperm concentration (100 million/ml) than three reference groups of blue collar workers (50–57 million/ml), who were enrolled as referents in earlier sperm studies.[1] Despite methodological limitations the findings seemed convincing and unexpected. The findings might be due to differences in behaviour, geographical factors, or general lifestyle including differences in dietary factors—for example, dietary intake of pesticides or food composition. Another study reported that members of two associations promoting the development of organic agriculture, and who had a diet consisting of at least 25% organic products, had a significantly higher sperm concentration (69 million/ml) than a control group of men working in an airline company (48 million/ml).[2] A clear dose-response association was not found.

If it is true that organic farmers as an occupational group have a high sperm count, it might be possible to unravel environmental or lifestyle determinants of male reproductive function. A large scale well designed study to examine the unexpected finding among organic farmers is highly warranted. The aim of our study was to confirm or refute the earlier reported finding of high sperm concentrations in organic farmers. To reduce the number of confounders related to lifestyle and profession, we compared semen quality in a group of organic farmers with a group of traditional farmers.

Methods

POPULATION

Samples of male traditional and organic farmers were selected in 1995–6 from registers in the Danish Ministry of Agriculture. A random sample was taken among traditional farmers from 37 municipalities in Jutland of those supposed to have agriculture as their main occupation (>20 hectares if they had animals; >70 hectares if they had no animals). A group of traditional farmers cultivating potatoes was identified through a potato flour factory. All the farmers were selected to obtain the shortest possible distance to the laboratory. An invitation with 12 questions was posted to 1124 farmers (775 traditional and 349 organic farmers), and 967 (86%) returned the questionnaire. A total of 331 were excluded because they were ineligible for the sperm study (farming not main occupation, or very small farm for the organic farmers in 1995 (n=165), vasectomy (n=102), age>50 (55) (n=26), not a farmer (n=26), moved or died (n=10), known azoospermia (n=2)). None was ineligible because of occupational exposure to lead, styrene, ionising radiation, microwaves, metal welding, mercury, or cadmium, or because of medical treatment with cytostatic drugs, salazopyrine, or anabolic steroids. Four men could not provide the semen sample and were excluded. Among the 789 eligible farmers, 256 (32.4%) agreed to provide semen samples. The participation rate was 28.8% among traditional farmers and 42.9% among organic farmers.

DATA COLLECTION

Semen was collected by masturbation, and we requested 2–7 days of sexual abstinence before the day of collection. Information on date, time, spillage, occurrence of fever >38°C, and

number of days since last ejaculation was recorded for each sample. The samples were analysed in a mobile laboratory[3] by one medical doctor and two trained technicians. The men were instructed to collect the sample within 1 hour before arrival of the mobile laboratory, and to keep the sample in a pocket close to the body.

Information on reproductive, medical, and occupational history and lifestyle habits was collected by a self completed questionnaire.

ASSESSMENT OF OCCUPATIONAL EXPOSURE TO PESTICIDES

Information about previous exposure to pesticides included total years working as a traditional or organic farmer, total number of years of exposure to pesticides, and last date of exposure. The semen samples were collected in the spring before the start of the spraying season. Characteristics of the participating farmers are shown in table 1.

LABORATORY METHODS

The semen samples were examined in the mobile laboratory to determine volume and sperm concentration. Video recordings and semen smears were prepared for later analysis of sperm motility, morphology, and vital scoring. The sample volume was measured in a graduated Falcon tube. The sperm concentration was measured with an improved Neubauer haemocytometer according to the WHO criteria.[4] An appropriate dilution was determined after a preliminary examination of the undiluted sample. Sperm were counted by a phase contrast technique at a magnification of 200. The sample was counted twice, and when there was >10% difference between the two counts, the sample dilution was remixed and the counting procedure repeated. Video recording was performed in a Makler chamber placed on a thermostat plate adjusted to 37°C (4.5 µl). The recordings were made on an undiluted sample (2 minutes).

Computer assisted analyses were undertaken with the Hopson sperm tracker system (firstly we analysed 25 s of recordings, and if at least 100 sperm cells were not tracked the duration was increased until 100 tracks were analysed). Curvilinear velocity (VCL) and straight line velocity (VSL) were initially chosen for analysis. The slides for morphology scoring were air dried, fixed in 96% ethanol, and stained with a modification of Papanicolaou's stain. Morphology was scored according to WHO criteria[4] (all performed by one technician), and, further, according to strict criteria[5] (performed by another technician). Vital staining was by the eosin-negrosin technique.

An internal quality control programme was established to evaluate the laboratory variation in assessment of different semen variables (the within technician coefficient of variation for sperm vitality was 11.3%; the between technicians coefficient of variation for sperm concentration was 9.5%, and for volume it was 4.6%. Furthermore, the laboratory participated in an external quality programme.[6] Morphology, and vitality scoring, computer assisted analysis, flow cytometric analysis of sperm chromatin structure, and hormone measurements were done blinded to the exposure group.

Table 1 Characteristics of the farmers in relation to occupational exposure

	Traditional farmers (n=171) n (%)	*Organic farmers (n=85) n (%)*
Have you ever sprayed with pesticides?		
Yes	170 (99.4)	57 (67.1)
When did you spray with pesticides the last time?		
Never sprayed		28 (32.9)
<1989		15 (17.6)
1990–3		14 (16.5)
1994		19 (22.4)
1995		9 (10.6)
Last spraying ⩽3 months before semen sample	9 (5.3)	
Last spraying 4–7 months before semen sample	110 (64.7)	1 (1.2)
Last spraying >7 months before semen sample	51 (30.0)	
Total number of spraying seasons:		
0	1 (0.6)	28 (32.9)
1–5	13 (7.6)	17 (20.0)
6–10	36 (21.2)	15 (17.6)
11–15	51 (30.0)	6 (7.1)
16–20	47 (27.6)	13 (15.3)
⩾21	22 (12.9)	6 (7.1)
Years as organic farmer (n):		
1		25 (29.4)
2–5		30 (35.3)
⩾6		30 (35.3)

Flow cytometric analysis of sperm chromatin structure

The chromatin structure of the spermatozoa was analysed with minor modifications according to the procedure described by Evenson *et al*.[7] Abnormal chromatin structure, defined here as increased susceptibility to acid induced denaturation in situ, is measured, after staining of sperm cells with acridine orange, by flow cytometric measurement of the metachromatic shift from green fluorescence (native DNA) to red (denatured, single stranded DNA) luminescence. The shift is expressed by αt, which is the ratio of red to total (red+green) fluorescence. After acidic denaturation, a higher proportion of single stranded DNA is expected in structurally altered than in normally condensed chromatin. In the sperm chromatin structure, αt is calculated for each sperm cell in a sample, and results are expressed as the mean (SD) of the αt distribution, and percentage of cells outside the main population (%COMPαt). Measurement of normal sperm produces a narrow αt distribution, but sperm with increased susceptibility to DNA denaturation have a broad distribution, and therefore, larger SDαt and %COMPαt values.

Reproductive hormones

Serum was stored at -20°C until hormone measurements, and all samples were analysed in the same laboratory. Serum concentrations of luteinising hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin were measured with DELFIA time resolved immunofluorometric assays from Wallac, Finland. The sensitivities of the LH, FSH, and sex hormone binding globulin assays were 0.05 IU/l, 0.06 IU/l, and 0.5 nmol/l, respectively. In these three assays the coefficients of variation within and between assays were <10%. Serum testosterone was measured with a radioimmunoassay from Diagnostic Products Corporation (DPC, Coat-a-Count, California,

Table 2 Characteristics of 256 farmers in a semen study

	Traditional farmers (n=171) n (%)	*Organic farmers (n=85) n (%)*
Person related characteristics:		
Age (y):		
<30	15 (8.8)	2 (2.3)
30–34	46 (26.9)	18 (21.2)
35–39	46 (26.9)	30 (35.3)
40–44	33 (19.3)	15 (17.7)
⩾45	31 (18.1)	20 (23.5)
Mean	38.0	40.3
SD	6.2	6.5
Body mass index, kg/m^2:		
Mean	25.4	24.9
SD	2.4	2.9
Smoking:		
1–10 cigarettes/day	11 (6.4)	6 (7.0)
>10 cigarettes/day	24 (14.1)	8 (9.4)
Pipe or cheroots	5 (2.9)	1 (1.2)
Non-smoker	131 (76.6)	70 (82.4)
Alcohol consumption (drinks/week):		
0	13 (7.6)	12 (14.1)
1–7	97 (57.1)	40 (47.1)
8–14	37 (21.8)	17 (20.0)
⩾15	23 (13.5)	16 (18.8)
Urogenital disorder*	14 (8.2)	15 (17.6)
Coital frequency times/week:		
<1	21 (12.7)	11 (12.9)
1	43 (26.1)	22 (25.9)
2	45 (27.3)	17 (20.0)
3	39 (23.6)	16 (18.8)
⩾4	17 (10.3)	19 (22.4)
Semen related characteristics:		
Duration of abstinence (days):		
⩽1.0	6 (3.5)	7 (8.2)
1.1–2.0	31 (18.2)	20 (23.5)
2.1–3.0	47 (27.7)	25 (29.4)
3.1–5.0	44 (25.9)	26 (30.6)
⩾5.1	42 (24.7)	7 (8.2)
Median	3.5	3.0
25–75 Percentile	2.5–5.0	2.0–4.0
Fever during the past 3 months (>38°C rectal)	13 (7.8)	12 (14.3)
Spillage at sampling	17 (10.2)	9 (11.1)
Minutes from sampling to start of analysis:		
⩽30	57 (33.3)	22 (25.9)
31–60	82 (48.0)	44 (51.8)
61–90	23 (13.5)	13 (15.3)
91–120	6 (3.5)	2 (2.3)
>120	3 (1.7)	4 (4.7)
Hour of blood sampling:		
Before 1200 am	77 (45)	50 (58.8)
After 1200 pm	94 (55)	35 (41.2)
Sampling months:		
February	30 (17.5)	
March	61 (35.7)	
April	61 35.7)	19 (22.4)
May	19 (11.1)	46 (54.1)
June		20 (23.5)

*Reported testicular cancer, cryptorchidism, orchitis, gonorrhoea, chlamydia infection, or syphilis.

USA). The sensitivity of the DPC testosterone assay was 0.23 nmol/l, and the coefficients of variation within and between assays were both <10%. Inhibin B was measured in an enzyme immunometric assay[8] that is specific for the bioactive inhibin B dimer (αβB). The sensitivity of the inhibin B assay was 20 pg/ml, and the coefficients of variation within and between assays were <12% and <17%, respectively.

ANALYSIS AND STATISTICAL METHODS

The farmers were divided into traditional or organic farmers according to the type of agriculture at the time of enrolment. Organic farmers cultivate without the use of pesticides, but two of them sprayed with pesticides at other places and were grouped as traditional farmers for the data analysis. Unadjusted mean or median values were calculated for the different sperm variables in the two groups. Multiple linear regression (SAS procedure GLM)[9] was used to compare differences between the two groups. To ensure that the underlying assumptions (normality of residuals and homogeneity of variances) were satisfied, some of the sperm variables were transformed. The sperm concentration and total count were transformed to third roots. The proportion of morphologically normal spermatozoa and the proportion of spermatozoa with other defects were transformed by the logit function. The logarithm was used to transform the %COMPαt (sperm chromatin structure) and FSH. Potential confounders were selected by their biological relevance irrespective of findings in this study, and included age (<35, 35–40, >40), semen spillage (yes/no), sexual abstinence (logarithm of days), fever during the previous 3 months (yes/no), smoking (yes/no), alcohol intake (<15, >15 drinks weekly), self reported reproductive disease (testicular cancer, cryptorchidism, orchitis, gonorrhoea, chlamydia infection, or syphilis: yes/no). Only age, genital disease, and hour of blood sampling (before or after 12 00 am) were included in the models concerning reproductive hormones. Adjusted group means of concentration and total count were calculated as the third power of the estimated means of the transformed variables with the reference group distribution of covariates. As proportions from the morphological scorings were logit transformed before analyses, the coefficients in the multiple regression measured effects on a logit scale. To facilitate the interpretation, these effects were expressed as odds ratios: the ratio of the odds for a normal cell from a traditional farmer to the odds for a normal cell from an organic farmer. The odds ratio was obtained as the exponential of the corresponding regression coefficient. A non-parametric test was used to test differences in volume.

Results

Characteristics of the participating traditional and organic farmers, and the characteristics related to semen are summarised in table 2. The traditional farmers were slightly younger and less often reported a urogenital disorder or fever during the past 3 months. The median abstinence period was slightly longer among traditional farmers.

Unadjusted median values and adjusted values of measures of semen quality and sex hormones for the two groups are presented in table 3. The unadjusted median sperm concentration for traditional and organic farmers was 58 million/ml and 64 million/ml, respectively. The 95% confidence interval (95% CI) on the adjusted group difference mean was (−14.8 to 14.2). No significant differences were found between the two groups in concentration, total count, proportion of non-vital spermatozoa, sperm chromatin structure, and motility variables. There was borderline significance between the two groups with respect to volume.

The traditional farmers had a significantly lower proportion of normal sperm heads according to WHO scoring (39.5% *v* 42.3%, p<0.01) and a lower proportion of normal spermatozoa according to the strict criteria (2.5% *v* 3.4%, p=0.02). No significant differ-

Table 3 Sperm variables and sex hormones among traditional and organic farmers

	Traditional farmers n=171	*Organic farmers n=85*	*p Value*
Volume (ml):*			
Unadjusted, median (25–75 percentile)	3.4 (2.5–5.1)	3.0 (2.4–3.9)	0.05
Concentration, millions/ml:			
Unadjusted, median (25–75 percentile)	58 (33 102)	64 (29 115)	
Adjusted, mean† (95% CI)	59.9 (46.6 to 75.6)	61.4	NS
Total count, millions:*			
Unadjusted, median (25–75 percentile)	221 (108–401)	202 (82–367)	
Adjusted, mean† (95% CI)	196 (146 to 255)	200	NS
Percentage non-vital spermatozoa:			
Median (25–75 percentile)	30.2 (22.6–42.3)	30.0 (21.7–40.5)	
Adjusted, mean† (95% CI)	33.9 (29.3 to 38.5)	33.8	NS
Percentage normal sperm heads (WHO):			
Median (25–75 percentile)	39.5 (33–45)	42.3 (37.0–49.0)	
Unadjusted, odds ratio (95% CI)	0.85 (0.77 to 0.95)	1.0	
Adjusted, odds ratio (95% CI)	0.83 (0.74 to 0.93)	1.0	0.001
Percentage with tail, midpiece, or cytoplasmic defects (WHO):			
Median (25–75 percentile)	12.5 (9.5–18.5)	14.0 (9.0–18.0)	
Unadjusted, odds ratio (95% CI)	0.98 (0.84 to 1.15)	1.0	
Adjusted, odds ratio (95% CI)	0.97 (0.83 to 1.13)	1.0	NS
Percentage normal spermatozoa (strict criteria):			
Median (25–75 percentile)	2.5 (1.0–4.3)	3.4 (2.3–5.3)	
Unadjusted, odds ratio (95% CI)	0.66 (0.48 to 0.92)	1.0	
Adjusted, odds ratio (95% CI)	0.67 (0.48 to 0.95)	1.0	0.02
Curved line velocity (VCL) μm/s:			
Unadjusted median (25–75 percentile)	79.5 (67.1–90.2)	78.4 (67.5–88.8)	
Adjusted mean† (95% CI)	79.3 (74.1 to 84.4)	80.2	NS
Straight line velocity (VSL) μm/s:			
Unadjusted median (25–75 percentile)	23.3 (18.7–27.3)	23.8 (20.6–31.3)	
Adjusted mean† (95% CI)	23.9 (21.5 to 26.3)	25.6	NS
SCSA:			
Unadjusted "mean(Xαt)" (median 25–75 percentile)	218.1 (209.9–230.0)	218.7 (209.1–236.7)	
Unadjusted %COMPαt (median 25–75 percentile)	12.1 (8.4–17.6)	12.9 (8.4–21.8)	NS
Testosterone/SHBG (units):			
Median (25–75 percentile)	0.42 (0.32–0.54)	0.44 (0.36–0.56)	
Adjusted mean‡ (95%CI)	0.43 (0.39 to 0.47)	0.48	0.02
FSH IU/l mean SD:			
Median (25–75 percentile)	3.6 (2.7–5.0)	4.1 (3.1–5.7)	
Adjusted mean‡ (95%CI)	3.7 (3.2 to 4.3)	4.2	NS
LH IU/l mean (SD):			
Median (25–75 percentile)	3.7 (2.9–4.7)	3.5 (2.4–5.2)	
Adjusted mean‡ (95%CI)	4.0 (3.5 to 4.5)	4.1	NS
Inhibin B (pg/ml):			
Median (25–75 percentile)	164 (126–210)	184 (150–237)	
Adjusted mean‡ (95%CI)	181 (161 to 201)	201	0.05

*Samples with spillage were excluded from the analysis.
†Adjusted mean calculated with covariate distribution as among reference group (organic farmers). Confounders included: age, urogenital disease, fever, spillage, time of abstinence, smoking, alcohol consumption. Time from masturbation to analysis was also included in the motility analysis.
‡Adjusted mean calculated with covariate distribution as among organic group. Confounders included: age, urogenital disease, hour of blood sampling.

ence (p=0.82) was found between the two groups in the proportion with tail, midpiece, or cytoplasmic defects. These findings were robust to adjustment for potential confounding factors.

REPRODUCTIVE HORMONES

Blood variables of the participating farmers are shown in table 3. Organic farmers had a higher serum inhibin B concentration (p=0.05) and testosterone/sex hormone binding globulin ratio (p=0.02), after control for age, genital diseases, and hour of blood sampling. No significant differences were found between the two groups in FSH and LH concentration.

Discussion

We found no difference between traditional and organic farmers in sperm concentration, total count, proportion of non-vital spermatozoa, sperm chromatin structure, and motility variables. The finding in two earlier cross sectional studies in Denmark of a higher sperm concentration in organic farmers and organic consumers than among other men was not confirmed.[1 2]

The traditional farmers had a significantly lower proportion of normal sperm heads according to two different morphology scorings (WHO and strict criteria) in two different laboratories. Collection of the semen samples before the start of the spraying season means that the farmers were not actually exposed, but 99% of the traditional farmers and 67% of the organic farmers had previously worked with pesticides. The results of the morphology scoring could be compatible with the hypothesis that long term exposure to pesticides reduces the proportion of normal spermatozoa. In a study among Danish greenhouse workers,[10 11] the level of estimated current exposure to pesticides was related to sperm morphology, but no relation was found to the number of years at work in ornamental greenhouses. The percentage of sperm with normal morphology declined by about 13% from a low exposure period to a high exposure period among greenhouse workers, whereas no decline was found in organic farmers. In our longitudinal study,[12] the farmers collected a second sample after the spraying season because we wanted to find out whether the result in the morphology was also present in this second sample. Table 4 shows that the traditional farmers had a significantly higher proportion of normal sperm heads according to WHO scoring, than the organic farmers (40.5% *v* 36.3%), whereas, according

Table 4 Morphology in first and second semen sample relative to exposure group

	Traditional farmers		*Organic farmers*	
	1st semen sample	*2nd semen sample*	*1st semen sample*	*2nd semen sample*
Percentage normal sperm heads (WHO), median (25–75 percentile)	39.5 (33.0–45.0)	40.5 (34.5–49.0)	42.3 (37.0–49.0)	36.3 (31.0–44.0)
Percentage with tail, midpiece or cytoplasmic defects (WHO), median (25–75 percentile)	12.5 (9.5–18.5)	16.0 (12.0–21.0)	14.0 (9.0–18.0)	15.8 (12.0–20.0)
Percentage normal spermatozoa (strict criteria), median (25–75 percentile)	2.5 (1.0–4.3)	2.5 (1.3–4.0)	3.4 (2.3–5.3)	3.0 (1.3–4.5)

*The second semen sample was collected from the same person after the spraying season.[12]

to strict criteria they had a lower but non-significant proportion of normal spermatozoa (2.5% *v* 3.0%). The almost opposite findings in the second sample weaken the conclusions based on the first semen sample of a real difference between the two groups. The opposite findings may also reflect the variability of the measurement itself.

The low participation rate might easily have introduced selection bias. We found[13] that the willingness to provide semen samples was higher among subfertile men, and furthermore, that the effect was modified by occupational exposure, resulting in a tendency to differential selection and possible biased risk estimates. Among traditional farmers the proportion providing semen samples was higher among subfertile men (OR 1.9 (95%CI 1.1 to 3.2)), but this trend was not found among organic farmers (OR 0.95 (95%CI 0.5 to 1.9)).[13] This selection bias might have introduced a bias away from the null hypothesis. The organic farmers may have an interest in reporting a high sperm quality and therefore less interest in participating if they suspected that their semen was poor. By contrast, subfertile traditional farmers might participate to a greater extent to obtain an evaluation of possible exposures in a hazardous workplace. The higher incidence of urogenital disorders in the organic farmer group may have caused a selection problem. However, the frequencies were small and urogenital disorders were included as confounders in the model.

Although we did not find any significant differences in sperm concentration and quality of the spermatozoa, the serum concentration of inhibin B—a marker of Sertoli cell function—was significantly lower in traditional farmers than in the organic group. This difference was not expressed by higher FSH concentrations, possibly due to an oestrogenic negative feed back effect of the pesticides, and may indicate slight impairment of spermatogenesis among farmers who used these compounds. Also the testosterone/sex hormone binding globulin ratio was significantly higher in organic farmers, which may be explained by slightly better Leydig cell function or increase of sex hormone binding globulin concentrations due to oestrogenic compounds. The findings that the organic farmers had a lower median abstinence period, a higher frequency of abstinence of >5 days, and a higher average frequency of coitus than the traditional farmers may explain some of the differences in reproductive hormones between the two groups. However, inclusion of frequency of coitus in the model did not change the results.

Lack of difference between the two groups could be due to previous exposure to pesticides among a group of the organic farmers, if historical exposure causes a cumulation of pesticides or irreversible damage. However, when the group of organic farmers who had never been working with pesticides were compared with the other farmers, no significant differences were found between the two groups, apart from a difference of borderline significance in morphology (strict criteria).

To reduce the number of confounders related to lifestyle and profession, we compared two groups of farmers. Despite that, there may be differences between the two groups in lifestyle, nutrition, exercise, etc. We adjusted for the most relevant confounders, and it is difficult to imagine that unmeasured risk factors could hide any true difference.

The sperm concentrations in the two groups (medians 58 *v* 64 million/ml) were comparable with those reported among organic consumers (median 69.0 million/ml),[2] and with those in some occupational groups studied in Denmark during recent years.[14] However, higher concentrations were found in the study of members of an organic farmers' association (median 100 million/ml)[1] and among greenhouse workers (median 83 million/ml).[10] The differences could be due to differences in participation rates (selection bias) or to distribution of another confounder. The participation rate in our study was low, and the participating groups may not truly represent the source population. Subfertile men and men with genital disorders are more willing to provide semen samples,[2 13] and in studies with a preferential participation of subfertile men, a higher average semen concentration might be expected with increasing participation rate. In the study of organic farmers and greenhouse workers, the participation rates were 74% and 62%, and this could explain some of the differences in sperm concentration.

The earlier finding of the high sperm concentration among members of an organic farmers' association (median 100 million/ml)[1] might also have been due to cluster sampling (participants in a weekend seminar), and it is important to take into consideration the low number of participants (n=30). The high concentration was found despite a low period of sexual abstinence (median 1.3 days).

Seasonal changes in sperm concentration have been described in many non-equatorial countries in the northern hemisphere,[15] with the lowest values in the three summer months, July, August, and September. The season of highest sperm concentration has not been clearly delineated. It has not been clarified whether the seasonal changes are due to changes in temperature, photoperiod (duration of daylight), an endogenous biological clock, or to a combination of these factors. The semen samples were collected from February to June, but the proportion of samples collected in June was higher among organic farmers (24%) than in traditional farmers (0%). If we suppose that seasonal changes lead to a lower concentration in June than in February, the time of collection in our study may have introduced a weak bias in favour of the null hypothesis. In the study among greenhouse workers and members of an organic farmers' association,[11] all the samples were collected during the winter, which also may have contributed to the differences in sperm concentrations compared with our study.

Statistical tests were carried out on 15 different semen variables, and it is important to take into consideration that there is a real danger that one comparison will be reported as significant, but that it might have arisen by chance. Although some significant proportions were found the actual magnitude of the differences was generally small, and the biological relevance of these small differences is probably of minor importance.

Conclusion

We compared a group of traditional farmers with a group of organic farmers and it was not possible to corroborate the earlier finding of higher sperm concentrations among organic farmers. Also, with the exception of higher concentrations of inhibin B in serum and testosterone/sex hormone binding globulin ratio in organic farmers, no differences between the two groups were found for reproductive hormones and other semen characteristics.

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Appendix: ASCLEPIOS

ASCLEPIOS is an EU biomedical concerted action research project dedicated to *occupational hazards to male reproductive capability*, coordinated by the Steno Institute of Public Health, University of Aarhus, Denmark, with the following participants: ● Belgium, Gent (P Kiss, A Mahmoud, M Vanhoorne, H Verstraelen) ● Denmark, Aarhus (A Abell, JP Bonde, S Brixen Larsen, G Danscher, E Ernst, H Kolstad; Copenhagen (A Giwercman) ● England, London (A Dale, M Joffe, N Shah) ● Finland, Helsinki (M-L Lindbohm, H Taskinen, M Sallmen); Turku (J Lähdetie) ● France, Paris (P. Jouannet, P Thonneau); Strasbourg (A Clavert) ● Germany, Erlangen (KH Schaller, W Zschiesche) ● Italy, Brescia (P Apostoli, S Porru); Milan (L Bisanti); Pietrasanta (L Lastrucci); Rome (M Spanò) ● Netherlands, Nijmegen (N Roeleveld, H Thuis, GA Zielhuis); Zeist (W de Kort) ● Poland, Lodz (K Sitarek) ●

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