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Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities

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BACKGROUND: In fertile populations, little is known about the association between semen parameters and time to pregnancy (TTP). METHODS: Pregnant women from Copenhagen, Edinburgh, Paris and Turku who conceived without medical intervention were asked for their TTP (942 couples), and their partners provided a semen sample. The proportion of morphologically normal sperm and the multiple anomalies index (MAI, ratio of the total number of anomalies to the number of abnormal sperm) were centrally estimated. We estimated rate ratios for the occurrence of a pregnancy by a discrete survival model, adjusted for sexual activity and female factors affecting fecundity. RESULTS: Increasing sperm concentration influenced TTP up to 55×10^6/ml. The proportion of morphologically normal sperm influenced TTP up to 39% according to David's criteria, and this association held among the subjects with a sperm concentration >55×10^6/ml. For strict criteria, the threshold value was 19% normal sperm. An increase of 0.5 in MAI was associated with an adjusted rate ratio for the occurrence of a pregnancy of 0.68 (95% confidence interval: 0.54–0.85). CONCLUSIONS: These results highlight the importance of sperm morphology parameters and indicate that the effect of proportion of normal sperm on TTP may be independent of sperm concentration.

Key words: fecundability/semen/sperm morphology/threshold values of semen characteristics/time to pregnancy

Introduction

The estimation of sperm concentration, motility and morphology is still the mainstay of the assessment of male reproductive health (World Health Organization, 1992, 1999), both when evaluating difficulties in obtaining a pregnancy and in reproductive toxicology or epidemiology. This is in spite of quite limited data on the diagnostic value of such criteria on the couple's ability to conceive (or fecundity).

Sperm concentration $<20\times10^6$/ml is associated with decreased fecundity (MacLeod and Gold, 1953; Rowe *et al.*, 1993), but one study indicated an association up to 40×10^6 sperm/ml (Bonde *et al.*, 1998a).

Sperm motility has also been found to be strongly associated with the probability of conception (MacLeod and Gold, 1953; Jouannet *et al.*, 1988; Larsen *et al.*, 2000). However, this parameter, when assessed subjectively, has a high inter-technician variation (Jørgensen *et al.*, 1997).

There are several ways to classify the morphology of sperm (Coetzee *et al.*, 1998). The 4th edition of the WHO manual (World Health Organization, 1999) recommends the use of the strict criteria (Menkveld *et al.*, 1990), but other similar classifications are also used (David *et al.*, 1975; World Health Organization, 1992). The proportion of normal sperm, when assessed by one of these three methods, is likely to influence the probability of fertilization or the probability of pregnancy (Mayaux *et al.*, 1985; Kruger *et al.*, 1986; Jouannet *et al.*, 1988; Ombelet *et al.*, 1997c; Bonde *et al.*, 1998a). The multiple anomalies index (MAI), or mean number of morphological anomalies per abnormal spermatozoon, has been shown to be associated with the probability of occurrence of conception among couples with fertility problems (Jouannet *et al.*, 1988).

These results on the influence of semen parameters on the probability of conception or pregnancy have been obtained from various situations and populations: couples attending an

IVF (Jeulin *et al*., 1986; Kruger *et al*., 1996; Coetzee *et al*., 1998) or an insemination programme (Mayaux *et al*., 1985; Ombelet *et al*., 1997b); in natural reproduction, populations including subjects monitored for fertility problems (Jouannet *et al*., 1988; Bostofte *et al*., 1990; Ombelet *et al*., 1997a) were mostly studied. In such studies, including both fertile and infertile couples, the associations observed between the probability of conception and the semen parameters may reflect differences in semen quality between sterile and fecund men. The generalization of these associations to the population of fertile couples is thus not straightforward. Fertile populations have rarely been studied, with the noticeable exception of one study which failed to detect any clear association between the proportion of normal sperm and the time taken to produce conception among 1130 husbands of pregnant women (MacLeod and Gold, 1953). To our knowledge, only one study examined the influence of semen parameters on the probability to conceive among couples not selected on the basis of their ability to conceive (Bonde *et al*., 1998a,b). This was a prospective study among first pregnancy planners, showing associations between time to pregnancy (TTP), an epidemiological indicator used to assess fecundity (Baird *et al*., 1986) and sperm concentration, motility, and the proportion of normal sperm assessed by WHO criteria (World Health Organization, 1992). Moreover, one study among healthy couples, excluding azoospermic men, showed an association between total sperm count, sperm concentration, the proportion of normal sperm on the one hand, and time to pregnancy on the other (Zinaman *et al*., 2000).

The debate on a possible decline in semen quality (Carlsen *et al*., 1992; Auger *et al*., 1995; Fisch and Goluboff, 1996; Irvine *et al*., 1996; Spira and Multigner, 1998; Swan *et al*., 2000) has increased the interest in the relationships between semen parameters and fecundity, in that they could shed light on the possible consequences of such a decline (Bonde *et al*., 1999). A precise understanding of the relationship between the conventional criteria of semen quality and the couples' fecundity in the general population would also be useful to establish the validity of the use of the waiting TTP as an epidemiological tool, allowing us to monitor trends in semen quality across time (Joffe, 2000), and to study the effect of environmental exposures on male reproductive health (Baird *et al*., 1986).

We studied the relations between semen parameters and TTP in a population of European pregnant couples.

Materials and methods

The general aim of the European study of partners to pregnant women was to compare the semen quality of partners to pregnant women between four European towns (Jørgensen *et al*., 2001). Companion articles based on the same data set compared the distribution of TTP between the four cities where the study took place (Jensen *et al*., 2001) and studied morphological defects of sperm (Auger *et al*., 2001).

Following demographers (Léridon, 1977; Wood, 1989), we denote here by 'fecundity' the biological ability to conceive, and by 'fertility' the fact of having achieved a pregnancy, which in this case lasted more than 3 months.

Recruitment

We recruited couples whose female partner had been pregnant for at least 3 months at the time of inclusion. Other eligibility criteria were: male partner being 20–45 years of age at the time of inclusion and born in the country in which he was currently living. Furthermore, the current pregnancy had to have been achieved by sexual relations, and not as a result of any fertility treatment. Diseases in reproductive organs or previous fertility treatment were not exclusion criteria for men or women. Pregnant women were invited to participate when they attended their first antenatal visit or 'parent-craft' class. Each woman was given written information if she was eligible, received self-administered questionnaires for both her and her partner, and was asked to book an appointment for her partner to deliver a semen and a blood sample and to have a physical examination performed. The man returned the questionnaires on the day of attendance or beforehand.

Couples were recruited over a full calendar year or more in each city from October 1996 to June 1998. In Copenhagen and Paris, the women were attending regular antenatal visits at Rigshospitalet and Hôpital Cochin respectively. The couples from Edinburgh were approached as they were attending 'parent-craft' classes run at the Royal Infirmary and Eastern general hospitals and outlying city general practitioner's clinics. In Turku, the women were contacted in the special maternity care units, which take care of most pregnant women. Except in Paris, the participants received economic compensation for their travel expenses, and lost working hours, according to local practices within this field. The study was approved by the local ethical committees and was performed in accordance with the Helsinki Declaration.

Questionnaires

The questionnaires were developed in English and translated into Danish, Finnish and French. The translated questionnaires were back-translated to English to control for translation errors. The questionnaires for the women included information on age at the start of the conception attempt, previous or current diseases in reproductive system, length and regularity of the menstrual cycle, use of contraceptive methods before the occurrence of pregnancy, frequency of sexual intercourse during conception attempt, reproductive history, height and weight before pregnancy, education and working conditions and exposures.

Definition of time to pregnancy

The TTP questions were phrased as in a previous validated study (Juul *et al*., 1999). The woman was asked: 'Were you and your partner doing anything to avoid pregnancy at the time you became pregnant?' If not, the woman was asked 'How many months did it take you/your partner to become pregnant? (From starting time until pregnancy)'. For women who became pregnant when the couple was not using a method to avoid pregnancy, we defined TTP as the number of months taken to conceive, from the woman's declarations. Answers 0 month were grouped together with 1 month. The TTP was censored after 13 months, which is the time when fertility treatment is often started; that is, for a couple achieving a pregnancy after 24 months, the only information used was a lack of pregnancy after 13 months of attempts. Since TTP cannot in this frame be defined for couples using a contraceptive method when pregnancy occurs, these couples were not included in the study.

Semen collection and analysis

The semen samples were collected by masturbation, generally in the laboratory premises. Some samples from Copenhagen and Turku were collected at home and immediately transported at body temperature to the laboratory.

In the four cities, the men were asked to respect a duration of sexual abstinence of 48 h, and the technicians in charge of the semen analysis were unaware of the questionnaire data, and in particular of the TTP.

Semen volume, sperm concentration and motility were measured in one laboratory in each city, as described previously (Jørgensen *et al*., 2001). A technician from each of the four laboratories took part in a standardization workshop in Copenhagen before the start of the study (Jørgensen *et al*., 1997). An external quality control of sperm concentration assessment was also conducted throughout the study, showing little evidence of systematic differences between laboratories (Jørgensen *et al*., 2001).

For sperm morphology assessment, the smears were made in each of the four laboratories from a 10 μl drop of semen, and were then mailed to Paris, where they were stained and analysed as described elsewhere (Auger *et al*., 2001). The proportion of morphologically normal sperm and the proportion of sperm presenting a given anomaly were evaluated by five well-trained technicians. The classification method of the anomalies of the sperm as described by David and colleagues (David *et al*., 1975) and later modified (Auger *et al*., 2001). A multiple entry system allowed us to record all anomalies of each observed spermatozoon, with a limitation of five anomalies per spermatozoon. This allowed us to estimate the multiple anomalies index (MAI), which is the mean number of anomalies per abnormal spermatozoon, irrespective of their location in the cell (Jouannet *et al*., 1988).

The slides were then sent to Turku, where an additional assessment of the proportion of morphologically normal sperm was subsequently undertaken on the same smears by one physician (AH), according to the strict morphology criteria (Menkveld *et al*., 1990).

Statistical analysis

The values of sperm concentration and MAI were dichotomized according to values from the literature (Jouannet *et al*., 1988; Rowe *et al*., 1993). The other semen parameters were dichotomized according to the round value closer to the 10th percentile of the distribution of the parameter (cf. Table III), which was an arbitrary choice. For each semen parameter, we estimated the unadjusted relative risk of occurrence of a pregnancy within 6 months of attempts by dividing the proportion of couples who conceived in ≤6 months in the group with a ‘poor’ value of the semen parameter by the equivalent proportion in the group with a ‘good’ value. These analyses were repeated among a sub-sample excluding couples with a low frequency of sexual intercourse, and female fertility disorders, to limit the potential confounding effect of those parameters on the estimated relative risks. We also categorized the concentration and morphology semen parameters in ten categories or more defined by round values of the parameter, and, for each of these categories, plotted the monthly probability of pregnancy (number of pregnancies/sum of all TTP, for couples belonging to this category) with its confidence interval against the mean semen parameter value. The probabilities of pregnancy were smoothed using locally weighted regression for binary data (Fahrmeir and Tutz, 1994) with the ksm function of Stata 6.0 statistical software (Stata corportation, College Station, TX, USA).

Adjusted analyses of TTP according to semen parameters

We used a survival model adapted to discrete survival times (Scheike and Jensen, 1997), as described in Appendix A. The model allows an estimation of the ratio of the instantaneous probabilities of conception for an increase by 1 unit of the semen parameter, or rate ratio. This model was fitted separately for each semen parameter.

The rate ratio can be interpreted as in a continuous Cox model (Scheike and Jensen, 1997), and is likely to vary the same way as the relative risk of pregnancy within 6 months of attempts described above, without being equivalent to it because it uses more information.

There might be subjects declaring as planned a pregnancy that resulted from a failure of birth control (Weinberg and Wilcox, 1998), which may bias our estimates; these pregnancies are likely to be declared as occurring in the first month of attempt. To test if the estimated associations could have been due to such a ‘definitional bias’, we excluded all first months of attempts at pregnancy and estimated once again the adjusted rate ratio of pregnancy associated with each semen parameter.

Adjustment factors

We adjusted for factors likely to influence the fecundity of the female partner independently of semen quality, so as to provide an estimate of the effect on TTP of variations in semen parameters when the female partner’s characteristics remained constant. Our logic of confounder selection was based on epidemiological practice (Rothman and Greenland, 1998). The adjustment factors were chosen either because they had an unadjusted influence on the probability of conception significant at the 20% level (variables thereafter indicated by *), or because they could have an influence on the probability of conception, as estimated from the literature (Baird *et al*., 1986). We did not adjust for male parameters likely to influence TTP (history of male urogenital disorder or male occupational factors for example) because we hypothesized that their action on TTP would be mainly mediated by alterations in semen parameters. We thus adjusted for: woman’s age at the beginning of the attempt at conception, cycle regularity*, previous history of female urogenital disorder* (all disorders were grouped into one binary variable, cf. Table II footnote for details), female tobacco consumption during attempt at pregnancy, woman’s educational level*, body mass index before conception [body wt (kg)/height (m^2)], previous use of oral contraceptive. We also adjusted for the duration of ejaculatory abstinence before semen collection*, city, technician performing the semen morphology analysis, since these factors were likely to influence our measure of the semen parameters, and for frequency of sexual intercourse during the attempt at pregnancy*. Models with semen volume, sperm concentration and total sperm count were also adjusted for the season of semen collection (Jørgensen *et al*., 2001). The way the variables were coded is indicated in footnotes to Tables II and IV. All categorical variables were coded by dummy variables (Hosmer and Lemeshow, 2000).

We did not systematically look for effect modifications (Miettinen, 1974; Rothman and Greenland, 1998) between these adjustment factors and semen parameters; only for the city of recruitment did we perform stratified analyses, that is, we estimated the association between TTP and the semen parameters separately for each city, and conducted thereafter an adjusted analysis to average the association over the four cities. As couples with and without a previous pregnancy may constitute different groups as far as their fecundity is concerned, we moreover estimated our statistical models separately in those two groups.

Threshold values of semen parameters

We estimated threshold values above or below which the variations of a given semen parameter associated with TTP ceased to have an effect on TTP, by means of ‘slope-threshold’ models described in the Appendix B.

Independent effect of the main semen parameters

To assess the question of the independence of the effects of the sperm concentration and morphology parameters on TTP, we estimated the rate ratios of pregnancy associated with the proportion of normal sperm in the subgroups of population with a high sperm concentration (above our estimated threshold value) or a lower sperm concentration. We also estimated the rate ratios of conception associated with sperm concentration, the proportion of normal sperm and MAI in a model in which those three parameters were simultaneously included and coded by the threshold variables defined above.

Results

Characteristics of the included subjects

Among the eligible couples who were given a questionnaire in Copenhagen, Paris and Turku, the proportion of men who delivered a semen sample ranged between 15 and 43%, according to the city. The number of invitations delivered in Edinburgh was not recorded, and the overall participation rate can therefore not be estimated. Sperm concentration or proportion of normal sperm were known for 1081 men, and TTP could be defined for 942 couples not using a contraceptive method when pregnancy occurred (87% of the included subjects), who constituted our final population under study (Table I). The mean value of TTP was 3.8 months, with a median of 2 months. TTP was censored for 79 of the 942 couples (8.3%) who conceived in $\geq$14 months.

The main semen parameters and other male characteristics are described in Table I for each city. The duration of sexual abstinence was shorter than 48 h for 105 men (11.4%). The proportions of men with a sperm concentration $\leq 20\times10^6$/ml sperm were 7.8% in the whole sample, and 6.8% among men with a duration of abstinence longer than 48 h. Each of the five technicians who performed the sperm morphology assessment in Paris analysed roughly the same proportion of semen samples among subjects with TTP smaller or greater than 7 months (data not shown; χ^2-test: $P = 0.91$). The mean proportion of morphologically normal sperm was 50.1 and 19.9% according to David's criteria and the strict criteria, respectively. The correlation coefficient between the two measures of proportion of normal sperm was 0.49 (847 men, $P < 0.00005$). MAI was negatively correlated with the proportion of normal sperm (David's criteria) and ln(sperm concentration), with correlation coefficients of –0.22 and –0.23 ($P < 0.00005$) respectively.

Female characteristics for couples with a TTP above or below 6 months are shown in Table II. The women with longer TTP were more often recruited in Paris than in the other cities, and had a greater chance of having a previous history of urogenital disorders. They also declared less frequent sexual intercourse during conception attempt, had a lower educational level, and more often had irregular menstrual cycles.

Relationships between TTP and semen parameters

We observed reduced probabilities of conception within 6 months of unprotected sexual intercourse if sperm concentration was $<20\times10^6$/ml, if proportion of normal sperm was <30% [David's criteria (Auger *et al*., 2001)] or 10% [strict criteria (Menkveld *et al*., 1990)]. If MAI was >1.6, or, less clearly, if the proportion of motile sperm was <50% (Table III). Similar findings were observed in the subpopulation of 558 couples with no previous history of female urogenital disorder, regular menstrual cycle, and more than one sexual intercourse per week during attempt at pregnancy. We found no reduced probability of conception if semen volume was <2 ml.

An increasing sperm concentration was linked with an increasing probability of conception after adjustment (Table IV); rate ratio of conception for subjects $<20\times10^6$/ml sperm compared to those $>20\times10^6$/ml was 0.68 (95% CI: 0.52–0.91). We observed no clearly decreased probability of conception for the subgroup of men with 20–60×10^6/ml sperm, compared to men with $>60\times10^6$/ml spermatozoa. The total sperm count was associated with the probability of conception, with a pattern of association similar to that estimated for sperm concentration. A low proportion of morphologically normal sperm was associated with a decreased probability of pregnancy with both classifications of morphology: the rate ratio associated with a 10% increase in the proportion of normal sperm was 1.08 with David's criteria (2827 months, 736 couples), and 1.05 when strict morphology criteria were used (2806 months, 747 couples). The MAI was also strongly related to the probability of conception, its higher values being associated with a decreased probability of conception.

Analyses adjusted for the factors previously mentioned and stratified according to parity indicated the following rate ratio of pregnancy, for primiparous and non-primiparous women respectively: 1.08 and 1.09 for ln(sperm concentration), 1.03 and 1.11 for proportion of normal sperm (David's criteria), 0.70 and 0.30 for MAI, coded as continuous variables as in Table IV.

We saw no evidence of a clear relationship between the probability of conception and either the proportion of motile sperm or semen volume.

When all first months of attempts at pregnancy were excluded from the analyses, the estimates were consistent with those reported in Table IV (data not shown).

Threshold effect of semen parameters on TTP

The unadjusted monthly probabilities of conception according to sperm concentration, total sperm count and sperm morphology parameters are shown in Figure 1. Apart possibly for MAI, the smoothed curves did not exhibit strictly monotonous trends.

For sperm concentration the threshold value, adjusted for the factors indicated in Table IV, was 55×10^6/ml (95% CI: 19–270×10^6/ml). The adjusted rate ratio of pregnancy for the 259 subjects with a sperm concentration between 20 and 55×10^6/ml, compared to the subjects with a sperm concentration $>55\times10^6$/ml, was 0.92 (95% CI: 0.77–1.09). For total sperm count, the estimated threshold value was 145×10^6 sperm (95% CI: 45×10^6 to infinity). The threshold values for the proportion of morphologically normal sperm were 39 and 19% with David's classification and the strict criteria, respectively (95% CI: 23–56 and 10–29%).

For MAI, we found no convincing evidence of a threshold effect and the linear method of coding the variable provided a better fit to the data than the threshold variable. That is, in our sample, variations in the MAI were monotonously associated with variations in TTP on the whole range of values of the MAI.

Table I. Number of contacted and included couples, time to pregnancy (TTP) and semen characteristics, according to city

City	Copenhagen	Paris	Turku	Edinburgh	Total
Number of eligible couples contacted	809	1368	1422	NA	NA
Men who delivered a semen sample (proportion among contacted couples)	348 (43.0)	208 (15.2)	275 (19.3)	250 (NA)	1081 (NA)
Use of a method to avoid pregnancy when the conception occurred[a]					
No	304 (87.4)	191 (91.8)	249 (90.5)	219 (87.6)	963 (89.1)
Yes	43 (12.4)	16 (7.7)	26 (9.5)	31 (12.4)	116 (10.7)
No answer	1 (0.3)	1 (0.5)	0 (0)	0 (0)	2 (0.2)
Couples with defined TTP[b]	302 (99.3)	191 (100.0)	237 (95.2)	212 (96.8)	942 (97.8)
Among men with defined TTP:					
Median TTP (proportion of couples who conceived within 3–6–12 months)	2 (55.8–71.8–79.9)	2 (51.0–72.6–82.7)	2 (57.5–71.6–77.5)	2 (54.4–70.4–78.8)	2 (55.0–71.6–79.6)
Mean man's age at the beginning of the attempt at pregnancy (years) (5–50–95th percentiles)	31 (25–30–39)	31 (25–31–39)	29 (22–29–38)	32 (25–32–38)	31 (24–30–38)
Previous history of urogenital disorder (proportion in %)[c]	63 (21.0)	45 (24.9)	61 (26.6)	54 (26.5)	223 (24.4)
Mean duration between the estimated date of conception and semen collection (weeks) (5–50–95th percentiles)	21 (17–21–30)	30 (19–30–42)	32 (18–32–41)	30 (14–34–41)	28 (17–27–40)
Season of semen collection (column proportion, %)					
Winter	55 (18.3)	49 (25.8)	60 (25.3)	63 (29.7)	227 (24.2)
Spring	77 (25.6)	50 (26.3)	79 (33.3)	44 (20.8)	250 (26.6)
Summer	63 (20.9)	36 (19.0)	36 (15.2)	49 (23.1)	184 (19.6)
Autumn	106 (35.2)	55 (29.0)	62 (26.2)	56 (26.4)	279 (29.7)
Sexual abstinence before semen collection (days) (5–50–95th percentiles)	3.4 (1.0–2.7–8.0)	6.5 (2.4–4.0–21.0)	4.6 (2.0–3.0–11.8)	6.9 (1.5–3.4–14.1)	5.2 (1.5–3.3–13.5)
Mean semen volume (ml) (5–50–95th percentiles)	3.9 (1.0–4.0–7.0)	4.2 (2.0–4.0–8.0)	4.2 (2–4–7)	4.0 (1–4–8)	4.1 (2–4–7)
Mean sperm concentration ($\times 10^6$/ml) (5–50–95th percentiles)	76 (12–60–178)	99 (17–78–232)	104 (18–81–259)	90 (19–76–192)	91 (15–73–229)
Total sperm count ($\times 10^6$) (5–50–95th percentiles)	279 (35–209–806)	406 (47–314–1228)	412 (54–324–1040)	349 (63–270–954)	354 (45–270–966)
Mean proportion of motile sperm (%) (5–50–95th percentiles)	60 (40–60–79)	56 (40–55–77)	66 (49–66–81)	67 (52–69–82)	62 (43–63–80)
Mean proportion of normal sperm (%), David's criteria (5–50–95th percentiles)[d]	49 (21–51–71)	50 (19–54–72)	52 (27–53–74)	49 (21–52–71)	50 (22–52–72)
Mean MAI (5–50–95th percentiles)[e]	1.60 (1.29–1.60–1.93)	1.51 (1.18–1.51–1.86)	1.63 (1.32–1.63–2.02)	1.58 (1.27–1.58–1.88)	1.58 (1.26–1.58–1.95)
Mean proportion of normal sperm (%), strict criteria (5–50–95th percentiles)[f]	20 (3–19–41)	23 (5–22–45)	19 (4–18–37)	19 (5–19–33)	20 (4–19–39)

[a]Proportion in per cent among the couples from the corresponding city for whom semen parameters are known.
[b]Proportion in per cent among couples who were not using a method to avoid pregnancy when the conception occurred.
[c]Previous history of mumps affecting the testicles, gonorrhoea, varicocele, of treatment for undescended testes, for varicocele, for inguinal hernia, for testicular torsion or testicular cancer, varicocele treatment, or diagnosis of hydrocele or varicocele during clinical examination.
[d]David: Classification of sperm morphological anomalies from (David *et al*., 1975; Auger *et al*., 2001).
[e]Mean number of anomalies per abnormal sperm (Jouannet *et al*., 1988).
[f]Strict criteria: classification of morphological anomalies with the strict criteria (Menkveld *et al*., 1990).
NA = not available; MAI = multiple anomalies index.

Table II. Characteristics of the 942 fertile European women with a known time to pregnancy

	Time to pregnancy		P^a
	1–6 months (n = 774)	⩾7 months (n = 168)	
Centre (%)			NS
Copenhagen (Denmark)	250 (82.8)	52 (17.2)	
Paris (France)	151 (79.1)	40 (20.9)	
Turku (Finland)	197 (83.1)	40 (16.9)	
Edinburgh (UK)	176 (83.0)	36 (17.0)	
Mean age and SD (years)	29.2 (4.1)	29.1 (3.7)	NS
Mean women's body mass index (5th to 95th percentiles, kg/m^2)	22.3 (18.3–28.7)	22.6 (18.1–31.8)	NS
Previous history of urogenital disorder (%)[b]	134 (17.7)	47 (28.1)	0.002
No. of women who smoked throughout the whole period of research of pregnancy (%)	229 (29.6)	59 (35.1)	NS
No. of primiparous women before the current pregnancy (%)	376 (48.8)	89 (53.0)	NS
Average frequency of sexual intercourse throughout the period of research of pregnancy (%)			
⩾7 times a week	57 (7.8)	4 (2.7)	<0.0005
2–6 times a week	486 (66.5)	84 (55.6)	
3 to 4 times a month	153 (20.9)	46 (30.5)	
⩽2 a month	35 (4.8)	17 (11.3)	
History of use of oral contraception (%)			
Yes	717 (92.6)	157 (93.5)	NS
Women's educational level (%)			0.02
No education	46 (6.0)	16 (9.6)	
Manual education	56 (7.3)	22 (13.3)	
Theoretical education	251 (32.9)	56 (33.7)	
University	318 (41.7)	57 (34.3)	
Still in education	92 (12.1)	15 (9.0)	
Regularity of bleeding intervals before occurrence of pregnancy (%)			<0.0005
Irregular	70 (9.4)	37 (23.4)	
Regular	417 (55.8)	110 (69.6)	
Regular due to pill	261 (35.1)	11 (7.0)	

Unless otherwise specified, the characteristics refer to the period of attempt at pregnancy. The numbers of women with missing data were <20 for each of the variables, except for frequency of sexual intercourse (60 women with missing data) and regularity of bleeding intervals (36 women with missing data).
[a]The P value gives the degree of significance of the differences between the groups with TTP shorter or greater than 6 months (χ^2-test or t-test for comparison of means). It was calculated excluding missing data.
[b]Infection of Fallopian tubes, operation on genital organs, pelvic infection or inflammation, endometriosis, fibroma in the uterus and appendicitis that ruptured.

Independent effects of sperm concentration and morphology on the probability of conception

The proportion of normal sperm exhibited similar associations with the probability of pregnancy when the sperm concentration was below or above 55×10^6/ml (Figure 2). The adjusted rate ratio of pregnancy associated with a 10% increase in the proportion of normal sperm (David's criteria) up to 39% (threshold variable) was 1.33 (95% CI: 1.06–1.66) among subjects with a sperm concentration $<55\times10^6$/ml, and 1.28 (1.04–1.57) among subjects with a sperm concentration $\geqslant55\times10^6$/ml. This is in favour of proportion of normal sperm having by itself an effect on the probability of occurrence of a pregnancy, even among subjects whose sperm concentration is above the levels likely to influence fecundity.

To take also into account MAI, we fitted one model including the proportion of normal sperm (threshold variable), the sperm concentration (threshold variable), and the MAI. The comparisons of the estimates of this model with those of models where each semen parameter was entered without the two others (Table V) tend to indicate that variations of sperm concentration, proportion of normal sperm and MAI are associated with variations in TTP even when the two other semen parameters remain constant.

Discussion

In this population of European couples who had recently conceived a pregnancy, the sperm concentration up to 55×10^6/ml, the proportion of morphologically normal sperm up to 39 or 19%, when it was assessed by David's criteria or by the strict criteria, respectively, and the MAI were related to TTP. We saw no clear association between the probability of pregnancy and either the ejaculate volume or the proportion of motile sperm.

Selection of the population under study

One important inclusion criterion of the study was a clinically recognised pregnancy lasting at least 3 months, which, in

Table III. Proportion and relative risk of conception within 6 months of attempts according to semen characteristics, among 942 fertile European couples

Semen parameter value	Whole population (942 couples)		Subpopulation (558 couples)[a]	
	No. (%) of subjects with TTP ⩽6 months	Relative risk and 95% CI[b]	No. (%) of subjects with TTP ⩽6 months	Relative risk and 95% CI[b]
Volume of ejaculate (ml)				
<2	29 (76.3)	0.93 (0.77–1.11)	18 (90.0)	1.02 (0.88–1.18)
⩾2*	744 (82.4)		475 (88.5)	
Sperm concentration ($\times 10^6$/ml)				
<20	52 (71.2)	0.86 (0.74–1.00)	30 (75.0)	0.84 (0.70–1.00)
⩾20*	721 (83.1)		463 (89.6)	
Total sperm count ($\times 10^6$ sperm)				
<70	62 (73.8)	0.67 (0.45–0.98)	38 (79.2)	0.89 (0.77–1.03)
⩾70*	696 (82.6)		445 (89.0)	
Proportion of motile sperm (%)				
<50	120 (77.4)	0.93 (0.85–1.02)	81 (84.4)	0.94 (0.86–1.03)
⩾50*	653 (83.1)		412 (89.4)	
Morphologically normal sperm (%), David[c]				
<30	74 (74.0)	0.89 (0.79–1.00)	46 (80.7)	0.90 (0.79–1.02)
⩾30*	644 (83.4)		411 (89.7)	
Multiple anomalies index[d]				
<1.6*	395 (84.8)	0.94 (0.88–1.00)	247 (90.2)	0.97 (0.91–1.03)
⩾1.6	323 (79.6)		210 (87.1)	
Morphologically normal sperm (%), strict criteria[e]				
<10	114 (79.7)	0.96 (0.88–1.05)	70 (85.4)	0.96 (0.87–1.05)
⩾10*	586 (83.1)		377 (89.3)	

*Reference category for the calculation of the relative risk.
[a]The sample is limited to the 558 couples whose female partner declared no previous history of urogenital disorder, having regular cycles, or regular due to oral contraceptives, and at least one sexual intercourse a week during the attempt at pregnancy.
[b]Rate ratio of conception within 6 months of attempts, calculated as the ratio of the proportions of subjects with time to pregnancy (TTP) ⩽6 months between the two categories. CI = confidence interval.
[c]David: classification of sperm morphological anomalies (David *et al*., 1975).
[d]Mean number of anomalies per abnormal spermatozoon (Jouannet *et al*., 1988).
[e]Strict criteria: classification of sperm morphological anomalies (Menkveld *et al*., 1990).

>95% of the cases end with a live birth (Kline *et al*., 1989). Infertile men are more likely to have poor semen quality than fertile men and they were not included in this study. This selection is likely to pull our estimations of the rate ratio of pregnancy towards 1, compared to studies with the attempt at pregnancy—as opposed to its achievement|being the inclusion criterion.

The participation rate was relatively low in the cities where it was estimated. The observed distributions of TTP and of the values of semen parameters may thus not be representative of those of couples achieving a pregnancy in the four cities or hospitals, but we cannot postulate any simple mechanism by which a selection bias could artificially strengthen the associations between TTP and some semen parameters. The low participation rate observed is still a limitation of our study, as in most studies implying a semen delivery.

We only defined TTP for pregnancies that occurred when the couple was not using any method to avoid pregnancy. When compared to the subjects with defined TTP, the 116 male partners of couples who conceived their pregnancy while using a method to avoid pregnancy had similar sperm concentration and MAI ($P > 0.7$, Student's *t*-test), but a smaller semen volume (3.7 versus 4.1 ml, $P = 0.06$) and a higher proportion of morphologically normal sperm (53.4 versus 50.1%, $P = 0.03$), indicating a possibly better male fecundity. If we assume that these couples would have conceived quickly, the fact that we could not take them into account is unlikely to have artificially strengthened the association between the probability of conception and the proportion of normal sperm. On the contrary, the exclusion of couples with unplanned pregnancies is likely to have pulled our rate ratio of pregnancy towards 1.

The city of origin of the participants was associated with some semen parameters (Jørgensen *et al*., 2001), and perhaps also with TTP (Juul *et al*., 1999; Jensen *et al*., 2001). Stratified analyses indicated that the associations between TTP and either sperm concentration or MAI had the same direction in the four cities: in Copenhagen, Paris, Turku and Edinburgh, the rate ratio of pregnancy associated with ln(sperm concentration) were 1.15, 1.31, 1.04 and 1.04 respectively, and the corresponding values for an increase by 0.5 in MAI were 0.76, 0.40, 0.84 and 0.74 respectively. For proportion of normal sperm (David's criteria), the rate ratio of conception had the same direction in three of the towns, and had a value very close to 1 in the fourth town (1.13, 1.20, 1.12 and 0.97, for Copenhagen, Paris, Turku and Edinburgh respectively). We hypothesized that these differences between cities in the rate ratio of pregnancy associated with the proportion of normal sperm were caused by random error, and therefore reported the results adjusted for the city and not the stratified results.

Table IV. Adjusted rate ratios of occurrence of a pregnancy, according to semen characteristics among 942 fertile European couples

Semen characteristic	No. of months of attempts at pregnancy	Adjusted rate ratio (95% CI)[a]
Volume of ejaculate (ml)		
Increase by 1 ml[b]	3137	1.00 (0.96–1.04)
⩽2	161	0.90 (0.59–1.38)
2–5	2399	1.00 (0.83–1.21)
⩾6	577	1[c]
Sperm concentration ($\times 10^6$/ml)		
ln(sperm concentration)[b,d]	3137	1.10 (1.00–1.20)
0–9	104	0.81 (0.49–1.33)
10–19	220	0.68 (0.48–0.96)
20–59	952	1.01 (0.83–1.22)
60–99	807	1.15 (0.95–1.39)
⩾100	1054	1[c]
Total sperm count ($\times 10^6$)		
ln(sperm count)[b,d]	3137	1.09 (1.01–1.19)
0–20	62	0.73 (0.36–1.48)
20–50	209	0.51 (0.34–0.77)
50–100	313	0.92 (0.68–1.25)
100–200	627	0.92 (0.73–1.17)
200–500	1304	0.95 (0.78–1.15)
⩾500	622	1[c]
Proportion of motile sperm (%)		
10% increase[b]	3137	1.00 (0.93–1.07)
<40	135	1.07 (0.90–1.27)
40–49	457	0.88 (0.69–1.13)
50–59	756	1.01 (0.67–1.53)
⩾60	1789	1[c]
Morphologically normal sperm (David's criteria) (%)		
10% increase[b]	2827	1.08 (1.03–1.15)
0–9	42	0.11 (0.02–0.81)
10–19	109	0.58 (0.35–0.94)
20–29	269	0.72 (0.52–0.98)
30–39	414	0.84 (0.65–1.07)
40–49	456	0.97 (0.78–1.21)
⩾50	1537	1[c]
MAI (David's criteria)		
Increase by 0.5[b]	2827	0.68 (0.54–0.85)
1–1.24	73	1.64 (1.05–2.58)
1.25–1.49	799	1.14 (0.94–1.39)
1.50–1.74	1270	1[c]
1.75–1.99	546	0.89 (0.71–1.12)
⩾2.00	139	0.63 (0.40–0.99)
Morphologically normal sperm (strict criteria) (%)		
10% increase[b]	2806	1.05 (0.98–1.13)
0–5	177	0.70 (0.49–1.01)
5–15	905	0.86 (0.70–1.06)
15–20	472	1.06 (0.85–1.33)
20–25	403	1.10 (0.86–1.40)
⩾25	849	1[c]

[a]Confidence interval. Adjusted for cycle number, previous history of female urogenital disorder, regularity of menstrual cycle, female cigarette smoking before attempt at pregnancy, woman's educational level, previous use of oral contraceptive, frequency of sexual intercourse (categorized as in Table II), woman's age at the beginning of the attempt at conception (19–24, 25–29, 30–34 and ⩾35 years), body mass index (<20, 20–25, ⩾25 kg/m^2), sexual abstinence before semen collection (< or ⩾48 h) and country. Rate ratio for semen volume, sperm concentration and total sperm count were also adjusted for the season of the year of sperm collection. Rate ratio for proportion of normal sperm and multiple anomalies index (MAI) were also adjusted for the technician who performed the semen morphology analysis.
[b]Included in the model as a continuous variable.
[c]Reference category for the estimations of the rate ratio.
[d]The rate ratio corresponds to the ratio of the probabilities of conception when the log-transformed semen parameter increased by 1, that is, when the semen parameter was multiplied by $e = 2.72$.

Semen parameters measurement

Semen parameters were measured from 3 to 9 months after the occurrence of the pregnancy. For some men, a factor or an exposure likely to decrease semen quality, thus inducing a long TTP, may have ceased before the semen delivery, therefore leading us to estimate the semen quality better than it actually was during the attempt at pregnancy. This potential classification bias is likely to have attenuated the existing associations between TTP and semen parameters, and not to have artificially increased their strength. The couples for which the man delivered a semen sample at the end of the pregnancy constitute a more homogeneous group, as far as the issue of the pregnancy is concerned, than those for which the semen sample was delivered in the fourth month of the pregnancy. We therefore estimated the rate ratios of pregnancy associated with sperm concentration and morphology parameters, restricting the population to the subjects who gave a semen sample between 20 and 40 weeks of gestation, and we obtained results very similar to those reported in Table IV.

Sperm concentration was reported to change with season (Jørgensen *et al.*, 2001), and the proportions of semen samples collected during each season were different in the four cities (Table I). Analyses stratified on the season of semen collection indicated that the directions of the association between probability of conception and sperm concentration were the same whatever the season, which tends to indicate that our adjustment for season in the analyses allowed us to control efficiently for the season of semen collection.

Female and male characteristics

Female characteristics and behaviours are major determinants of a couple's fecundity (Baird *et al.*, 1986), and some of them, like previous history of sexually transmitted diseases, may moreover be associated with the semen parameters. Such female factors are thus likely to confound the association between TTP and semen parameters. The adjustment for female parameters assessed by questionnaire, not through biological measurements, may not be fully efficient to prevent confounding, but it was the only information on female factors available in our study.

We did not adjust for male history of genital pathology, as its effect on the probability of pregnancy would mainly be mediated through semen parameters, which were the variables of interest. Similarly, we did not adjust for the parity, since it is a characteristic of the couple that could be influenced by the semen parameters (Rothman and Greenland, 1998). A stratification of the results on woman's parity (Results section) tended to show that sperm concentration and MAI had the same effects on TTP in primiparous and non-primiparous couples, coherent with the effect estimated regardless of parity. However, proportion of normal sperm assessed by David's criteria was more strongly associated with TTP among non-primiparous couples than among primiparous couples.

Relationships between TTP and semen parameters

Our results confirm that a sperm concentration $<20\times 10^6$/ml is associated with decreased fecundity (Rowe *et al.*, 1993),

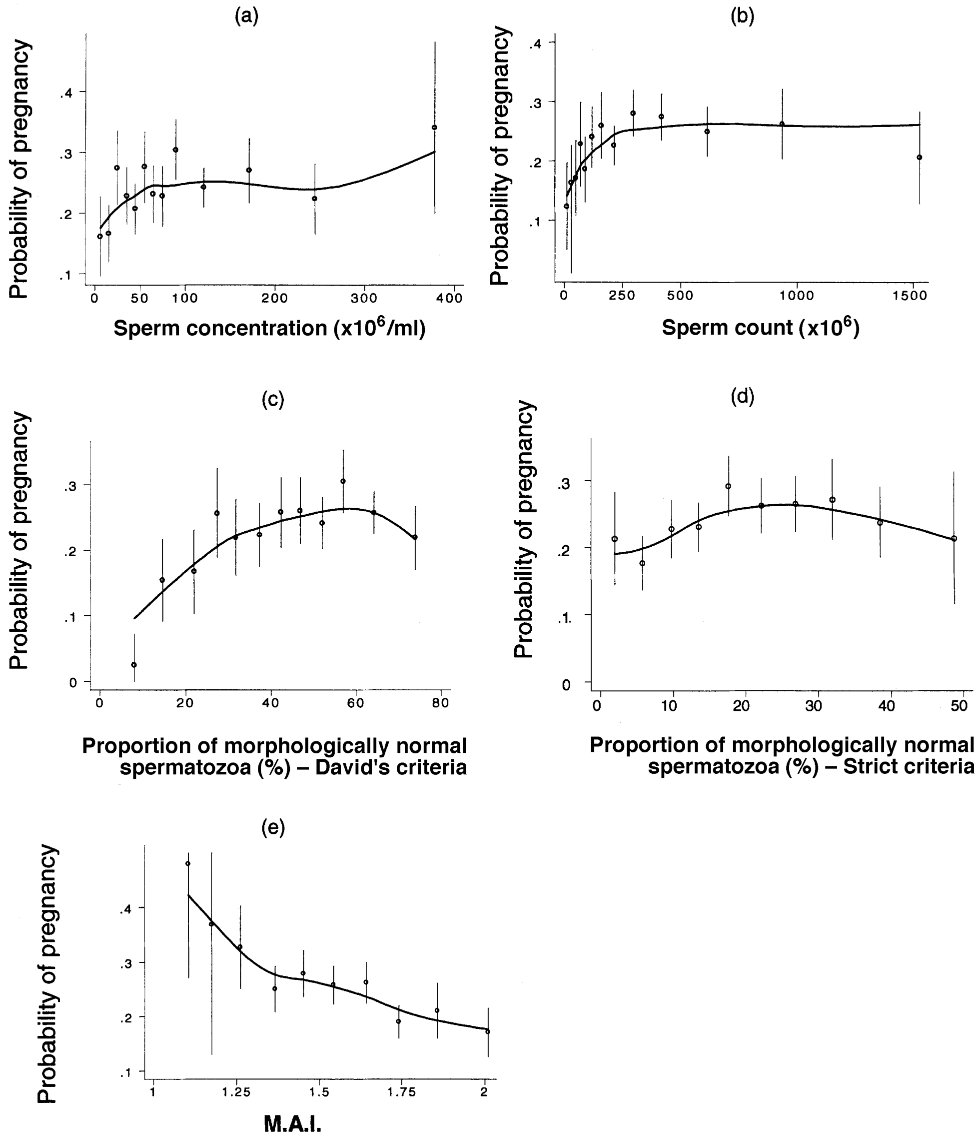

Figure 1. Unadjusted probability of pregnancy per month of attempt at pregnancy according to semen parameters, estimated in a population of 942 fertile European couples. (**a**) Sperm concentration, (**b**) total sperm count, (**c**) proportion of morphologically normal sperm assessed using David's criteria, (**d**) proportion of morphologically normal sperm assessed using strict criteria, and (**e**) multiple anomalies index (M.A.I.). The circles indicate the proportion of months in which a clinically detected pregnancy began, among the couples with the corresponding semen parameter value. The vertical bars indicate the 95% confidence intervals of these point estimates. A locally weighted regression model was fitted to the points (continuous curves). Note that the vertical axis scales vary between curves.

and indicate that sperm concentration could influence TTP up to 55×10^6/ml (95% CI: 19–270×10^6/ml), a value corresponding to the 35th percentile of the distribution of sperm concentration among the subjects included in the study. This is not inconsistent with the fact that we observed no clearly decreased fecundity for the 259 subjects with a sperm concentration between 20 and 55×10^6/ml, compared to subjects with a sperm concentration $>55\times10^6$/ml (rate ratio = 0.92; 95% CI: 0.77–1.09). In a study on partners of pregnant women who obtained their pregnancy without medical assistance (MacLeod and Gold, 1953), the median time taken to conceive was shorter among subjects with a sperm concentration $>40\times10^6$/ml than among subjects with a sperm concentration between 20 and 39×10^6/ml. A study among first pregnancy planners (Bonde *et al.*, 1998a), which used statistical methods similar to ours, also indicated that sperm concentration could indeed influence fecundity up to 40×10^6/ml.

The estimated association of total sperm count with TTP was quite similar to that of sperm concentration, with a threshold value of 145×10^6 sperm.

We observed an increase in the probability of pregnancy with proportion of normal sperm, up to 39% normal sperm according to David's classification of anomalies and 19% according to the strict morphology criteria. A similar association was observed in the study of first pregnancy planners (Bonde *et al.*, 1998a), in which another classification system of sperm morphology was used (World Health Organization, 1992), as well as in a study comparing couples who conceived in less than one year of attempt at pregnancy to couples attending an infertility clinic (Ombelet *et al.*, 1997a), in which

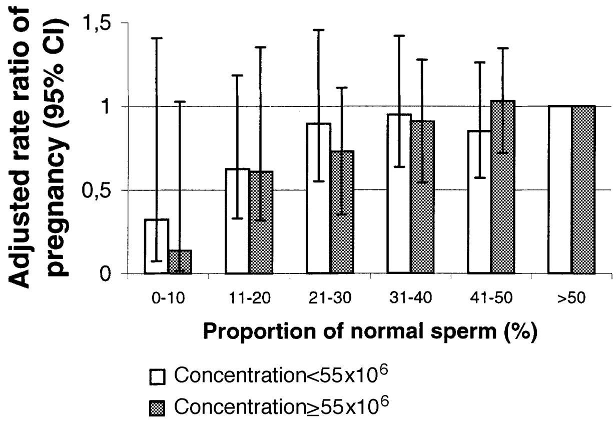

Figure 2. Adjusted rate ratios of pregnancy according to proportion of normal sperm (David's criteria) coded in six categories (subjects with >50% normal sperm taken as a reference), for subjects with sperm concentration below (1012 months of attempts at pregnancy) and above (1815 months) 55×10^6/ml. Rate ratios are indicated by the histograms, and the vertical bars show the corresponding 95% confidence intervals (CI).

Table V. Comparison of the association between time to pregnancy (TTP) and sperm concentration, proportion of morphologically normal sperm (David's criteria) and multiple anomalies index (MAI), in models where each semen parameter is taken into account without the two others (2nd column), and where all three semen parameters are entered simultaneously (3rd column). All the rate ratios of pregnancy were adjusted for the factors mentioned in Table IV, including season of sperm collection and technician in charge of the sperm morphology analysis. n = 2826 months of attempts at pregnancy

Variable	Rate ratio and 95% confidence interval	
	Each semen variable at a time (three different models)	All three semen parameters simultaneously (single model)
Sperm concentration[a]	1.24 (1.05–1.46)	1.10 (0.92–1.31)
Proportion of normal sperm[b]	1.34 (1.16–1.56)	1.23 (1.04–1.46)
MAI[c]	0.67 (0.54–0.84)	0.82 (0.63–1.05)

[a]Coded as slope threshold variable, with threshold at 55×10^6/ml. The rate ratio is given for an increase by 1 in ln(concentration) as long as concentration remains $<55\times10^6$/ml.
[b]Coded as slope threshold variable, with threshold at 39% normal sperm. The rate ratio is given for an increase of 10% of the proportion of normal sperm, as long as it remains <39%.
[c]Continuous coding without threshold. The rate ratio is given for an increase by 0.5 in MAI.

sperm morphology was assessed by strict criteria. In a study among healthy couples, including only women with regular menstrual cycles (Zinaman *et al.*, 1996, 2000), the probability of conception was associated with the proportion of normal sperm assessed by the strict criteria up to a threshold of 8%, a value smaller than the value of 19% reported here. In a sample of cycles of artificial insemination with donor semen (Mayaux *et al.*, 1985), it was reported that the proportion of normal sperm affected the probability of conception below a threshold of ~60% (David's criteria).

Both classifications of morphological anomalies used in this study exhibited qualitatively similar associations with TTP, although they do not define normal and abnormal sperm in the same way. We will soon undertake a comparison of the two morphology classifications used in this study.

We found moreover that the proportion of normal sperm, up to 39% normal sperm (David's criteria) was associated with the probability of pregnancy among subjects with a sperm concentration $>55\times10^6$/ml. This association was adjusted for potential female confounding factors, duration of sexual abstinence and frequency of sexual intercourse during the attempt at pregnancy. This indicates that the association observed between TTP and proportion of normal sperm was very unlikely to be due to the association between TTP and sperm concentration. A similar trend was reported in another study using another classification of morphological anomalies, for subjects with a sperm concentration $>40\times10^6$/ml, and without adjustment for potential confounding factors (Bonde *et al.*, 1998a).

The relatively high threshold values that we reported for sperm concentration and morphology do not imply that subjects have a higher probability of a clinically significant reduced fecundity as soon as one of their semen parameters is below the threshold; for example, the average fecundability of a group of men with a sperm concentration close to 30×10^6/ml may be 80–90% of the fecundability of a group of men with a sperm concentration of 80×10^6/ml. In other words, these threshold values should not be considered as values discriminating efficiently couples likely to need a long time to conceive from couples likely to conceive quickly. This question can be assessed by other methods (Menkveld *et al.*, 2001).

The MAI proved to be significantly related to the probability of occurrence of a clinically recognized pregnancy. Its variations were associated with variations in TTP on the whole range of values of MAI, without any clear threshold pattern.

We observed no convincing relationships between either ejaculate volume or motility and TTP. Since ejaculate volume is assessed with a good consistency between several laboratories (Jørgensen *et al.*, 1997), and since there is no strong evidence of an association between ejaculate volume and TTP in the literature (MacLeod and Gold, 1953; Bonde *et al.*, 1998a), the association between semen volume and TTP is likely to be weak among fertile couples.

Several studies have shown relationships between TTP or duration of infertility, and the proportion of motile sperm cells in various populations (Ducot *et al.*, 1988; Jouannet *et al.*, 1988; Bostofte *et al.*, 1990; Eimers *et al.*, 1994; Bonde *et al.*, 1998a). In our study, sperm motility was assessed in four different laboratories with relatively poor inter-laboratory consistency (Jørgensen *et al.*, 1997). When examining the association between proportion of motile sperm and probability of pregnancy separately in the four cities, it appeared that increases in the proportion of motile sperm were associated with an increased probability of conception in two cities (adjusted rate ratio for a 10% increase = 1.12, 95% CI: 1.00–1.24 in Copenhagen, and 1.15, 95% CI: 0.97–1.36 in Turku), whereas the association was very weak or in the direction opposed to the one expected in the two other cities (rate ratio = 1.03, 95% CI: 0.87–1.23 in Edinburgh, and 0.84, 95% CI: 0.71–1.01 in Paris). This is in favour of a lack of efficient standardization of the methods of estimation of the proportion of motile sperm between the cities.

In conclusion, in a population of partners of pregnant women, sperm concentration, proportion of normal spermatozoa assessed by David's and by the strict criteria, and MAI were clearly associated with the TTP of the couple. The morphology parameters proved to be strongly associated with TTP, in particular MAI, which deserves more systematic assessment when evaluating semen characteristics. The association between proportion of normal sperm (David's criteria) and the probability of conception existed independently of sperm concentration. Importantly, the relationships that we found between sperm concentration and morphology on the one hand, and fecundity on the other, existed at relatively high values of the semen parameters. This study, including no sterile men, tends to show that variations in sperm concentration and morphology affect fecundity in a continuous manner, and not only as a trigger inducing sterility.

Although these results shed some light on the biological mechanisms of human ability to conceive, one must also insist on the relatively poor predictive value of the semen parameters taken into account in this study: among the 76 couples who needed >12 months to conceive, only 37% had a poor semen quality (defined as a sperm concentration $<20\times10^6$/ml, or a proportion of morphologically normal sperm <30% using David's criteria, or a MAI >1.8). When restricting the study population to the couples declaring a frequency of sexual intercourse of one per week or more, and whose female partner had regular menstrual cycles the year before conception, the proportion of men with a poor semen quality among those who needed >12 months to conceive was 44% (95% CI: 39–49%). This relatively low sensitivity tends to indicate that the conventional semen parameters assessed in this study (excluding semen motility) are of limited value to detect couples needing more than one year to obtain a pregnancy, and confirms that many other male or female factors are indeed implied in the biological mechanisms of human reproduction.

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Appendix A. A discrete survival model to analyse TTP data

The TTP of a given couple i (i = 1, ..., n) was represented by binary variables, each variable corresponding to 1 month of attempt at pregnancy and indicating whether an effective fertilization occurred during this month of attempt at pregnancy (coded 1) or not (coded 0). These binary variables (Y_{it}, t = 1 to TTP of couple i, i = 1, ≈, n) were analysed by means of binomial regression, with a complementary log-log link function:

$$\ln[-\ln(1 - P_{it})] = \alpha_t + X_i\beta + Z_i'\gamma \qquad (1)$$

where P_{it}, the expectation of Y_{it}, is the probability of occurrence of a pregnancy for couple i during month t ($t \leqslant 13$), conditionally on the absence of pregnancy during the first $t - 1$ months of attempts. X_i represents the value of the semen parameter under study for man i, and Z_i' is the horizontal vector of adjustment variables of the couple.

α_t, β, γ, were estimated by the maximum likelihood method (Stata, glm function). This model was fitted separately for each semen parameter. The value of exp(β) corresponds to the ratio of the instantaneous probabilities, or incidence rates of conception when the semen parameter X increases by 1 unit, also called rate ratio, adjusted on the other variables coded by Z, as in a continuous Cox model (Scheike and Jensen, 1997).

Appendix B. The slope-threshold model

For the sperm concentration (log transformed), total sperm count (log transformed), and morphology parameters, we generated threshold variables, i.e. variables equal to the parameter under (respectively above) a threshold value, and equal to the threshold value if the parameter was greater (respectively smaller) than the threshold. That is, is X stands for proportion of normal sperm, and we created the threshold variable X_T, where T is the tested threshold value:

$$X_T = X \text{ if } X < T$$
$$X_T = T \text{ if } X \geqslant T$$

For each semen parameter, we included the threshold variable in a model taking into account all adjustment factors, and evaluated the deviance of the model. The creation of the threshold variable, the adjustment of the model and deviance estimation were repeated for each possible threshold value, that is, on the range of variation of the corresponding semen parameter. Our estimation of the value of the threshold was that corresponding to the model with the smallest deviance (Rothman and Greenland, 1998, p. 410). A 95% CI for the estimated threshold value was calculated, with limits being the values of the thresholds corresponding to the models with a deviance equal or immediately greater than that of the model with the estimated threshold plus 3.84 (95th percentile of a χ^2 distribution). This estimation procedure was described elsewhere in the case of linear regression (Hinkley, 1971). Since it models the association between TTP and each semen parameter by a continuous function made of two linear segments, this method may be more sensitive to detect a threshold than the method consisting of coding the semen parameter into several groups and looking for statistically significant differences in the probabilities of conception between groups, which corresponds to a modelling of the association by a step function.

As a confirmation of the results given by this method, we also plotted the smoothed probability of pregnancy as a function of each semen parameter (data not shown). This was obtained by means of a general additive model, written like model (1) in which the variable X coding for the semen parameter was replaced by a more general function of the semen parameter (Fahrmeir and Tutz, 1994). Adjustment factors were entered as in the final model, and the model's parameters were estimated with the gam function from S-Plus statistical software (Mathsoft, Seattle, WA, USA).