



HAL
open science

Semen quality of young men in Switzerland a nationwide cross-sectional population-based study

R Rahban, L Priskorn, A Senn, E Stettler, F Galli, J. Vargas, M van den Bergh, A Fusconi, Ronan Garlantezec, T K K Jensen, et al.

► To cite this version:

R Rahban, L Priskorn, A Senn, E Stettler, F Galli, et al.. Semen quality of young men in Switzerland a nationwide cross-sectional population-based study. *Andrology*, 2019, 7 (6), pp.818-826. 10.1111/andr.12645 . hal-02153625

HAL Id: hal-02153625

<https://hal-univ-rennes1.archives-ouvertes.fr/hal-02153625>

Submitted on 15 Jul 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives | 4.0 International License

ORIGINAL ARTICLE

Correspondence:

Serge Nef, Department of Genetic Medicine and Development, University of Geneva, CH-1211 Genève, Switzerland.
E-mail: Serge.Nef@unige.ch

*These authors contributed equally to this work as senior authors.

†Individual members of the NICER Working group are listed in the acknowledgments section.

Keywords:

geographic variations, lifestyle factors, semen quality, sperm count, testicular cancer, young men




Received: 19-Dec-2018

Revised: 11-Mar-2019

Accepted: 10-Apr-2019

doi: 10.1111/andr.12645

Semen quality of young men in Switzerland: a nationwide cross-sectional population-based study

¹R. Rahban, ²L. Priskorn, ¹A. Senn, ^{1,3}E. Stettler, ⁴F. Galli, ⁵J. Vargas, ⁶M. Van den Bergh, ⁷A. Fusconi, ⁸R. Garlantezec, ²T. K. Jensen, ⁸L. Multigner , ²N. E. Skakkebaek, ⁵M. Germond, ^{2,*}N. Jørgensen , ^{1,*}S. Nef  and [†]the NICER Working Group

¹Department of Genetic Medicine and Development, University of Geneva, Geneva, Switzerland, ²Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, ³Swiss Armed Forces Joint Staff, Medical Services, Ittigen, Switzerland, ⁴National Institute for Cancer Epidemiology and Registration (NICER), Zürich, Switzerland, ⁵Centre de Procréation Médicalement Assistée SA, Fertas SA et Fondation FABER, Lausanne, Switzerland, ⁶Kinderwunschzentrum, Kantonspital Baden, Baden, Switzerland, ⁷Centro Cantonale di Fertilità, Ospedale di Locarno La Carità, Locarno, Switzerland, and ⁸Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) – UMR_S 1085, Université de Rennes, Rennes, France

ABSTRACT

Background: Sperm counts have been steadily decreasing over the past five decades with regional differences in the Western world. The reasons behind these trends are complex, but numerous insights indicate that environmental and lifestyle factors are important players.

Objective: To evaluate semen quality and male reproductive health in Switzerland.

Materials and methods: A nationwide cross-sectional study was conducted on 2523 young men coming from all regions of Switzerland, recruited during military conscription. Semen volume, sperm concentration, motility, and morphology were analyzed. Anatomy of the genital area and testicular volume was recorded. Testicular cancer incidence rates in the general population were retrieved from Swiss regional registries.

Results: Median sperm concentration adjusted for period of sexual abstinence was 48 million/mL. Comparing with the 5th percentile of the WHO reference values for fertile men, 17% of men had sperm concentration below 15 million/mL, 25% had less than 40% motile spermatozoa, and 43% had less than 4% normal forms. Disparities in semen quality among geographic regions, urbanization rates, and linguistic areas were limited. A larger proportion of men with poor semen quality had been exposed *in utero* to maternal smoking. Furthermore, testicular cancer incidence rates in the Swiss general population increased significantly between 1980 and 2014.

Discussion: For the first time, a systematic sampling among young men has confirmed that semen quality is affected on a national level. The median sperm concentration measured is among the lowest observed in Europe. No specific geographical differences could be identified. Further studies are needed to determine to what extent the fertility of Swiss men is compromised and to evaluate the impact of environmental and lifestyle factors.

Conclusion: A significant proportion of Swiss young men display suboptimal semen quality with only 38% having sperm concentration, motility, and morphology values that met WHO semen reference criteria.

INTRODUCTION

One of the first studies demonstrating a decline in sperm count over a fifty years' time period was published in the nineties (Carlsen *et al.*, 1992). The causes of the increasing number of men with low semen quality remained, however, marginally addressed, in part because of advancements reached concomitantly by medically assisted reproductive technologies (Palermo

et al., 1992). A quarter of a century later, a meta-analysis evaluating temporal trends in sperm count confirmed earlier findings and reported a continuous significant decrease in sperm concentration in the Western world for the past twenty years (Levine *et al.*, 2017).

The observed decrease in semen quality is more likely to be related to environmental factors rather than genetics. Some

studies aiming at evaluating the impact of environmental and lifestyle factors on semen quality have been conducted on fertile men or partners of infertile women. Swan *et al.* (2003) evaluated differences in semen quality of fertile men in four American states with different urbanization levels (Missouri, California, Minnesota, and New York). Reduced semen quality was observed in semirural and agricultural areas, the highest median sperm count being in the state of New York with zero agriculture surfaces. A study on male partners of infertile women consulting fertility clinics in France showed that the lowest sperm concentrations were in regions with high agricultural surfaces (Le Moal *et al.*, 2014). These studies highlighted the importance of environmental factors, but a potential selection bias in the choice of the study population was often discussed whereby results were thought not to necessarily reflect the situation in the general population (Muller *et al.*, 2004; Hauser *et al.*, 2005; Deonandan & Jaleel, 2012).

Another set of studies on populations where men had no previous knowledge of their fertility status were conducted in several European countries, the USA, and Japan. Subjects were young men from the general population, aged 18–24 years, and often recruited in the context of military conscription. These studies reported median sperm concentration of 41–67 Mio/mL and revealed important regional differences in semen quality underlining once more the relevance of environmental or lifestyle factors (Andersen *et al.*, 2000; Jorgensen, 2001, 2002; Punab *et al.*, 2002; Richthoff *et al.*, 2002; Tsarev *et al.*, 2005; Paasch *et al.*, 2008; Mendiola *et al.*, 2011, 2013; Fernandez *et al.*, 2012; Iwamoto *et al.*, 2013; Erenpreiss *et al.*, 2017; Priskorn *et al.*, 2018; Rodprasert *et al.*, 2019). However, most of these studies were conducted in one single, often urban, region, leading to results that cannot necessarily be generalized to represent the situation in the whole country. The impact of geographical variations within one country cannot be evaluated in such a study design. Nevertheless, the incidence of testicular cancer in the previously mentioned studies has frequently been shown to correlate with low semen quality trends (Serrano *et al.*, 2013; Skakkebaek *et al.*, 2016).

The main purpose of the present work was to carry out the first standardized nationwide cross-sectional study on young men from the general population in order to evaluate semen quality in Switzerland and determine the impact of geographical factors. The incidence rate of testicular cancer was also extracted from regional registries in order to further describe the male reproductive health in Switzerland.

MATERIALS AND METHODS

Study population

Over 97% of all young Swiss men, aged between 18 and 22 years, are asked to attend a mandatory 2–3-day military camp prior to their potential enrollment into the army. Six recruitment centers distributed among the main Swiss cantons (states) are located in Lausanne (Vaud), Windisch (Aargau), Monteceneri (Ticino), Rütli (Zürich), Sumiswald (Bern), and Mels (St-Gallen) (Fig. S1). These centers were involved sequentially in the sampling process over a period spanning from September 2005 to June 2017. For each canton, the number of required participants was calculated proportionally to the resident male population (16–60 years). All enlisted conscripts of a given center received a detailed description of the study, a consent form, and two questionnaires—one for themselves and one for their parents. A total

of 92,274 men were contacted and 4837 (5.3%) sent back these documents completed with personal information, of which 2734 (3%) contributed biological material to the study (Fig. S2). These volunteers were given an appointment to undergo a physical examination and provide a semen sample in a nearby andrology laboratory. No exclusion criteria were applied during data collection; however, only men gestated in Switzerland (2523 out of 2734) were included in the current analysis. Study participants received a gift worth the equivalent of 50 Euros at the time of the physical examination. Ethical approval was obtained according to the requirements in the cantons of Vaud (17-01-2005, 01/02), Zürich (EK-StV-Nr. 27-2006), Ticino (Rif.CE 1886) and Geneva (2016-01674).

Questionnaires

Participants completed a questionnaire covering personal information divided into four sections: (i) Residence and general health information—such as previously diagnosed diseases and pharmacological drug intake during the 3 months immediately prior to participating in the study; (ii) health of their urogenital system such as occurrence of varicocele, as well as records on diagnosed and treated malformations such as cryptorchidism and hypospadias; (iii) lifestyle and diet—such as alcohol consumption and smoking; (iv) education. The mothers completed a separate questionnaire focusing on the time period close to conception, fetal life, and birth of the study volunteers.

Physical examination and sample collection

Trained urologists assessed the anatomy of the genital area, i.e., the presence of surgical scars, hypospadias, cryptorchidism, and varicocele. The testicular volume was measured by palpation using Praders orchidometer and/or by ultrasound (Logic 400 CL, GE Healthcare, Glattbrugg/ZH, Switzerland), and gynecomastia was evaluated by palpation. A significant correlation was observed between the two types of testicular volume measurement, but as expected values recorded with ultrasound were lower (al Salim *et al.*, 1995; Sakamoto *et al.*, 2007). A correction factor of 1.5 was determined using Bland–Altman analysis and was applied to these measures. Tanner stages of pubic hair were recorded from one to six, as previously described (Garn, 1956). Varicocele was determined according to the international grading system; grade 0 representing the absence of varicocele and grades 1 to 3 representing increasing severity of the enlarged vessels, as previously described (Dubin & Amelar, 1970; Kim & Goldstein, 2008). Grade 1 was notably determined after the Valsalva maneuver. Military physicians measured the men's weight and height during their visit to the recruitment centers, and BMI was calculated (kg/m^2). Participants were asked to provide a semen sample obtained by masturbation in a private room at the andrology laboratory after a recommended period of sexual abstinence of at least two days. The ejaculate was collected in a sterile and non-toxic 15-mL tube mounted with a funnel (Spermo-Sampler, Verridial, Blonay VD, Switzerland).

Semen analyses

Date and time of the last ejaculation were recorded, and the abstinence period was calculated. Semen volume was determined by weighing the tube before and after collection. Samples were incubated for 20–40 min at 37 °C to allow liquefaction and then thoroughly homogenized using a disposable Pasteur

pipette. Sperm concentration was assessed using the Computer Assisted Sperm Analyzer (CASA, Sperm Class Analyzer—SCA, MICROPTIC, Barcelona, Spain), and dilutions were made using a commercialized HEPES buffered with 0.4% HSA sperm medium (IVF basics, Gynotec B.V, GC Malden, The Netherlands) when needed. Aliquots (5 μ L) of the diluted sample were transferred in a 20- μ m-deep counting chamber (Leja Products, GN Nieuw-Vennep, The Netherlands) that was placed on a thermostatic (37 °C) microscope stage. Observations were made with a 10 \times phase contrast objective at a final 100 \times magnification. For each sample, a minimum of 400 sperm cell tracks were captured at a rate of 25 images per second using the CASA. Sperm concentration and kinetic parameters were recorded and the video sequences stored. For sperm morphology analysis, smears on glass slides were air-dried and sent to a central laboratory where they were Papanicolaou-stained. One hundred and fifty sperm cells were captured for each volunteer and automatically classified as normal or abnormal by an algorithm that was developed by Microptic based on the stricter criteria following WHO guidelines (Menkveld *et al.*, 1990). The majority of the slides were analyzed using the CASA system ($n = 2218$), however some slides ($n = 101$) were analyzed by a single trained technician conforming to the evaluation standards of the CASA with a high correlation between the two methods of assessment.

Quality assurance and quality control

A set of standard operating procedures (SOPs) were established at the beginning of the data collection. Every collaborating andrology laboratory applied the same protocols for semen analysis according to the guidelines dictated by the World Health Organization in 1999 (sample collection, initial macroscopic examination, and microscopic investigations). Sperm concentration, motility, and kinematic parameters were determined using the CASA system in order to avoid interpersonal variations in assessing concentration and motility. Identical CASA systems were used in all study locations, and very strict instructions were followed for recording the sequences such as frame rate, sperm concentration, and counting chamber depth. All the video sequences were then carefully examined and validated by two trained technicians throughout the whole study period. Internal quality control for the CASA assessment was performed before each session using latex beads of known concentrations (QC beads, BioScreen, New York, NY, USA), and external quality control for the technician's assessment was regularly performed to evaluate sperm concentration, motility, and morphology (UKNEQAS: <https://ukneqas.org.uk>, EQConline : <https://www.eqconline.org>).

Statistical analysis

Medians with the 5th and 95th percentiles were used to describe continuous variables, and frequencies were employed to describe categorical variables. Descriptive results are shown for the entire population as well as after stratification of men in three groups according to the measured sperm concentrations: <15 Mio/mL, 15–40 Mio/mL, and >40 Mio/mL. The first cutoff was chosen based on the 5th percentile published by the WHO for fertile men (Cooper *et al.*, 2010). The second cutoff was chosen based on consensus between several 'time to pregnancy' (TTP) studies where the TTP was shown to increase for sperm concentration up to a threshold of 40–50 Mio/mL (Bonde *et al.*, 1998; Guzick *et al.*, 2001; Slama *et al.*, 2002). Differences between the three groups

were tested using the Kruskal–Wallis test for continuous variables and chi-square for categorical variables. Linear regression analysis was carried out to obtain adjusted semen values corrected for confounding factors (see below). To correct for skewed distribution, sperm concentration and total sperm counts were normalized by a cubic root transformation and sperm motility was normalized by natural logarithmic transformation. Volume and sperm morphology were included untransformed. Duration of abstinence (standardized to 96 h) was taken into account for values of semen volume, sperm concentration, and total sperm count. The time between the delivery of the semen sample and the start of motility analysis (standardized to 30 min) was taken into account for the percentage of motile spermatozoa. No seasonal variation or any effect of the collection site could be detected for the semen parameters. Further adjustments for explanatory factors such as lifestyle factors (BMI, smoking, alcohol consumption) or genital malformations were not performed in order to represent the situation as it is in the cohort. However, semen parameters were separately evaluated for a subgroup of 1115 men who were not exposed to maternal smoking *in utero* and who were not diagnosed with varicocele or cryptorchidism. Men were stratified in groups according to their place of living in (i) the three geographic regions characteristic of the Swiss territory (Jura, Plateau, and Alps), (ii) the three urbanization rates (urban, intermediate, and rural), and (iii) the linguistic regions (French, Italian, and German), in order to explore potential effects of geographical and lifestyle factors on the observed semen quality. Data on geographic regions, urbanization categories, and linguistic areas were obtained from the Office of Federal Statistics (OFS) (Viktor Goebel, 2014; OFS, 2016) and cross-linked with the residence data in our questionnaires. A p -value below 0.05 was considered statistically significant. Data were analyzed using a commercially available package (IBM SPSS Statistics 23, NY, USA).

Testicular cancer incidence trend analysis in Switzerland

Incidence trend analysis was performed on testicular cancer data obtained from the National Institute for Cancer Epidemiology and Registration (NICER), which combines data from cantonal cancer registries. Data registered for at least five years from the last available diagnosis year (2014) were selected. Rates were age-standardized with the direct method by using the European standard population of 2013. Annual percentage change (APC) in incidence rates for cases pooled bi-annually to reduce variability was estimated with the Joinpoint Regression Program (version 4.6.0.0, August 2018; Statistical Research and Applications Branch, American National Cancer Institute). Completeness of case ascertainment and data quality among Swiss cancer registries have been recently assessed without detecting signs of over- or under-registration (Lorez *et al.*, 2017).

RESULTS

Basic description of the study population

The general characteristics and reproductive parameters of the study population are summarized in Table 1. The majority of the study participants was healthy, had an educational level higher than obligatory school (ending at age 15 in Switzerland), and did not have prior knowledge of their fertility status. These traits did not differ according to groups of sperm concentration (Table 1; A). Similarly, no significant differences were observed

Table 1 Basic description of the total number of men and of groups of men stratified according to their sperm concentration. Results are presented as medians (5th–95th percentiles) for continuous variables or percentages for categorical variables. Testicular volume is represented as mean (\pm SD)

	N with data	Total population (N = 2523)	Sperm concentration (Mio/mL)			p-value ^f
			Group 1 <15 (N = 425, 17%)	Group 2 15–40 (N = 683, 27%)	Group 3 >40 (N = 1415, 56%)	
A: General characteristics						
Age (years)	2523	19 (18–21)	20 (18–21)	19 (18–21)	19 (18–22)	0.4
Height (cm)	2267	179 (169–190)	178 (169–189)	179 (168–189)	179 (169–190)	0.8
Weight (kg)	2269	72 (59–93)	73 (59–94)	72 (59–95)	72 (58–92)	0.9
BMI (kg/m ²)	2267	22.6 (18–29)	22.5 (19–29)	22.6 (18–29)	22.6 (18–28)	0.8
Self Reported Health – excellent or very good (%)	2164	97.5	97.5	97.3	97.5	0.9
Medication last 3 months (%) ^a	2150	9.4	11.6	8.7	9.1	0.3
Ever fathered a child (%)	2144	1.5	1.7	1.7	1.4	0.8
Experienced fertility problem (%) ^b	2127	0.1	0.3	0	0.1	0.6
Educational level, higher than obligatory school (%)	2155	85.8	85.0	86.3	85.8	0.8
B: Lifestyle factors						
Cigarette smokers (%)	2156	26.3	26.0	30.2	24.6	0.03
Cigarettes/day, smokers only	568	10 (1–20)	10 (1–23)	10 (1–20)	7.8 (1–20)	0.02
Alcohol consumers (%) ^c	2156	83.0	80.0	85.0	83.0	0.2
Alcohol, consumers only (units/week)	1789	5.0 (1–24)	5.0 (1–18)	5.0 (1–25)	5.8 (1–25)	0.1
C: Parental parameters						
Maternal BMI at conception (kg/m ²)	1511	21.2 (18–26)	21 (18–27)	21 (18–26)	21 (18–26)	0.5
Maternal age at conception (years)	1656	29 (23–37)	30 (23–38)	29 (22–37)	29 (23–37)	0.5
Paternal age at conception (years)	1646	32 (25–43)	32 (25–44)	32 (25–42)	32 (25–43)	0.9
Mother smoked during pregnancy (%)	1657	13.0	18.6	12.6	11.5	0.008
D: Previously diagnosed/treated						
Asthma (%)	2089	10.4	9.6	10.6	10.6	0.9
Other diseases (%) ^d	2031	11.7	12.8	10.7	11.8	0.6
STD (chlamdia &/or gonnorrhoea) treated (%)	2144	0.7	0.9	1.0	0.6	0.6
Cryptorchidism treated (%)	1698	3.8	8.5	4.8	1.9	<0.001
Varicocele operated (%)	2133	1.0	2.0	0.3	1.0	0.04
E: Physical examination						
Gynecomastia (%)	1373	6.8	5	6.5	7.6	0.5
Pubic hair, stage 5–6 (%)	1386	86.3	83.7	86.6	87.0	0.4
Hydrocele (%)	1411	2.4	2.0	2.1	2.7	0.7
Varicocele (%)	1413	18.0	26.4	18.2	15.3	<0.001
Testicular volume, mean \pm SD (mL) ^e	1406	17.5 (10–25)	16.2 (10–25)	17.0 (10–25)	18.5 (10–25)	<0.001
F: Semen parameters						
Ejaculation abstinence (days)	2523	2.8 (1.5–6.8)	2.8 (1.1–5.7)	2.8 (1.0–6.0)	3.0 (1.7–7.0)	<0.001
Volume (mL)	2523	2.8 (1.0–5.6)	2.8 (0.7–5.7)	3.0 (1.3–5.7)	2.8 (1.1–5.5)	<0.001
Sperm Concentration (Mio/mL)	2523	47 (3.5–178)	7 (0–14)	27 (16–39)	81 (43–218)	–
Total sperm count (Mio)	2523	128 (7.7–524.5)	17 (0–58)	78 (30–178)	226 (77–680)	<0.001
Motile sperm (%)	2523	53 (18.2–83.4)	39 (0–72)	53 (24–75)	58 (26–86)	<0.001
Normal morphology (%)	2319	4 (0–17)	3 (0–14)	4 (0–15)	5 (0–18)	<0.001

^aTaken any medication during the 3 months immediately prior to participating in the study. ^bUnable to conceive a child despite their willingness. ^cSum of intake of beer, wine and strong alcohol in recent weeks prior to participation in the study. ^dSuffering from autoimmune diseases and/or cancer and/or diabetes and/or hepatitis and/or hypertension and/or thyroid. ^eMean of the right and left testicular volumes measured with Prader's Orchidometer and/or ultrasound. A correction factor of 1.5 was applied to correct for under-estimated values measured with ultrasound. ^fp-value for comparison of results between semen quality categories. Kruskal–Wallis test has been used for continuous variables and chi-square test for categorical variables. A p-value below 0.05 was considered statistically significant and was highlighted in bold.

in maternal or paternal age and maternal BMI at the time of conception between the three groups (Table 1; C). In contrast, more men with concentration < 15 Mio/mL had been exposed *in utero* to maternal smoking (Table 1; C). With regard to previously diagnosed reproductive disorders, a higher proportion of men with concentration < 15 Mio/mL had been treated for cryptorchidism (Table 1; D). Varicocele (grade 1 to 3) also occurred more frequently among those men, whose mean testicular volume was also lower (Table 1; E).

Semen quality of young men in Switzerland

The median sperm concentration and total sperm count were 47 Mio/mL and 128 Mio, respectively (Table 1; F). When adjusted to a period of abstinence of 96 h, the median sperm concentration was 48 Mio/mL (Table 2). Comparing with the

reference level set by the WHO for fertile men, 17% of men had sperm concentration below 15 Mio/mL and 17% had less than 39 million as total sperm counts in their ejaculate (Fig. 1A,B). In terms of motility, 25% displayed low values with less than 40% motile sperms and 43% had less than 4% morphologically normal sperm cells (Fig. 1C,D). Overall, sixty-two percent had one or more semen parameters that fell below the WHO thresholds (Fig. 1E). The adjusted median sperm concentration was only slightly higher after exclusion of men with known reproductive disorders and exposure to maternal smoking (Table 2).

Stratification of men according to geography, urbanization rate, and linguistic region

We evaluated geographic differences in sperm concentration across the three major landscapes in Switzerland according to

Table 2 Adjusted semen parameters of total population and subgroup of men not diagnosed with varicocele, treated for cryptorchidism, or having been exposed *in-utero* to maternal smoking. Adjusted results are presented as medians (5–95% confidence interval) calculated by linear regression analysis

	Adjusted values ^a	
	Total population (N = 2523)	Subgroup (N = 1115)
Ejaculation abstinence (days)	2.8 (1.5–6.8)	2.9 (1.5–7)
Volume (mL)	3 (2.9–3.1)	3 (2.9–3.3)
Sperm concentration (Mio/mL)	48 (42–55)	51 (49–60)
Total sperm count (Mio)	136 (121–145)	151 (124–173)
Motile sperm (%)	51 (50–52)	50 (48–51)
Normal morphology (%)	5 (0–17)	5 (0–17)

^aVolume, sperm concentration and total sperm count are adjusted to a period of abstinence of 96 h; sperm motility is adjusted to 30 min between delivery of semen sample and start of motility analysis. Morphology was unadjusted and median is presented with 5–95th percentiles.

the place of living and of gestation. A lower proportion of men living in the Jura had low sperm concentration, and the difference was significant between Jura and Plateau with 9% vs. 17%, respectively (Fig. 2A and Table S1). When evaluating the relation to urbanization, there were no differences among the three sperm concentration groups (Fig. 2B and Table S2). Upon stratification according to the linguistic regions as a proxy for lifestyle factors (German- or French-Italian-speaking regions),

there were also no differences in semen parameters (Fig. 2C and Table S3).

The incidence of testicular cancer in Switzerland

In order to complete the description of the male reproductive health status in Switzerland, we evaluated the occurrence of testicular cancer in the general population according to cantonal registries. The age-adjusted incidence rates of testicular cancer were shown to have been steadily increasing over the past 35 years from 7.6 cases in 1980 to 10.4 per 100,000 in 2014 (Fig. 3).

DISCUSSION

In this standardized nationwide cross-sectional study, we describe semen quality at a national level for the first time. We found that the adjusted median sperm concentration (48 Mio/mL) is among the lowest in Europe. When compared to the WHO reference levels, 62% of men had semen parameters associated with a prolonged waiting time to pregnancy (Guzick *et al.*, 2001; Cooper *et al.*, 2010). An evaluation of geographical factors, urbanization rates, or linguistic regions revealed no major differences in semen quality.

An important matter to primarily address concerns the relatively low participation rate (5.3%) of the invited subjects. More than 97% of Swiss young men were invited to participate in the study accounting for 92,274 individual over a twelve years’ time period. A self-selection bias cannot be excluded, but because the aim of our study is to evaluate male reproductive health, we first evaluated the presence of potential bias with this respect. We found that 99.9% of the volunteers had no prior knowledge of

Figure 1 Frequency distribution of basic semen parameters of Swiss men. (A) 17% of cases have oligozoospermia with sperm concentration below 15 Mio/mL, (B) 17% have less than 39 million spermatozoa in their ejaculate, (C) 25% have asthenozoospermia with less than 40% motile spermatozoa, and (D) 43% have teratozoospermia with less than 4% morphologically normal spermatozoa. (E) Pie chart illustrating the number of men with a combination of parameters below WHO reference values; 62% of men has at least one value below the thresholds set for fertile men. Gray columns indicate the percentage of men with values below the WHO thresholds (A–C: n = 2523; D: n = 2319).

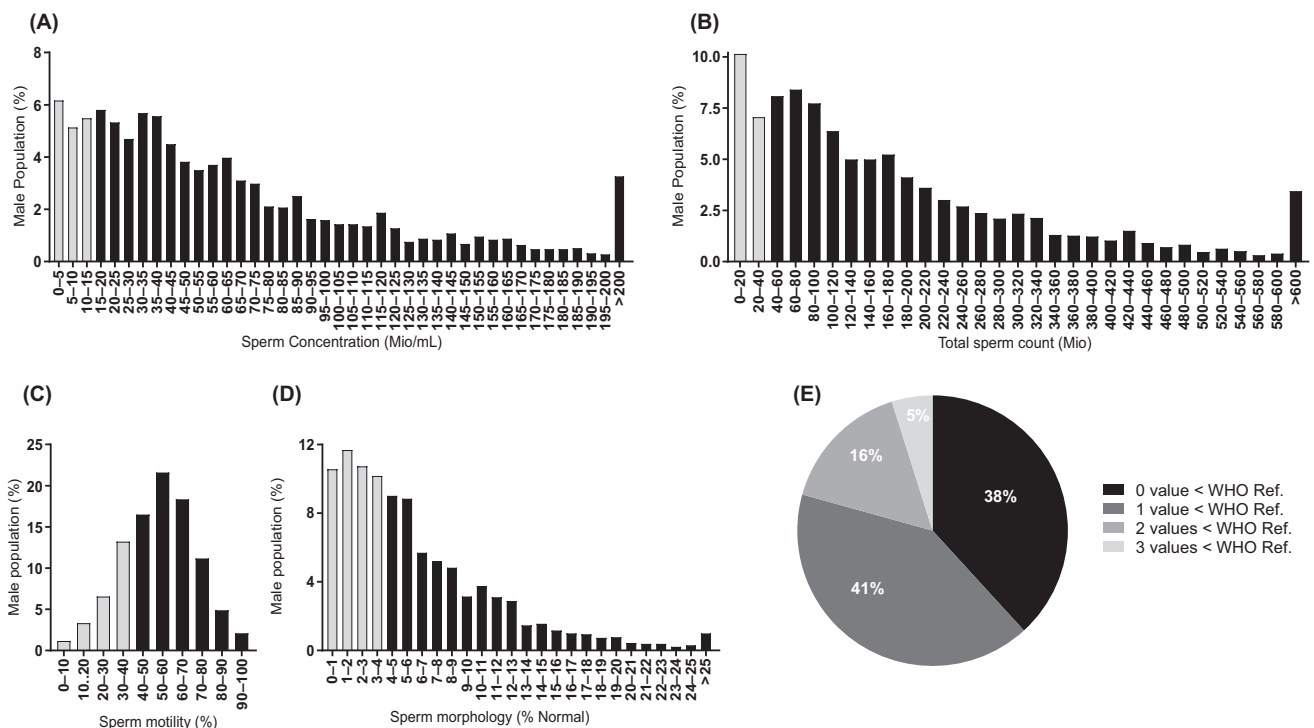


Figure 2 Minor geographic variations in semen quality in Switzerland. (A) Stratification of men according to their place of living in the three main geographical regions revealed that there are only slight variations in the proportion of men with sperm concentration <15 Mio/mL (Jura, Plateau, Alps). (B) Attribution to each man to an urbanization category shows that there are no differences between the three sperm concentration groups. (C) Stratification according to the linguistic regions as a proxy for lifestyle factors also indicates no variation in sperm counts in the German-speaking and French-Italian-speaking regions. *p*-value for comparison of results between sperm concentration categories was calculated using chi-square test (significant when *p* < 0.05).

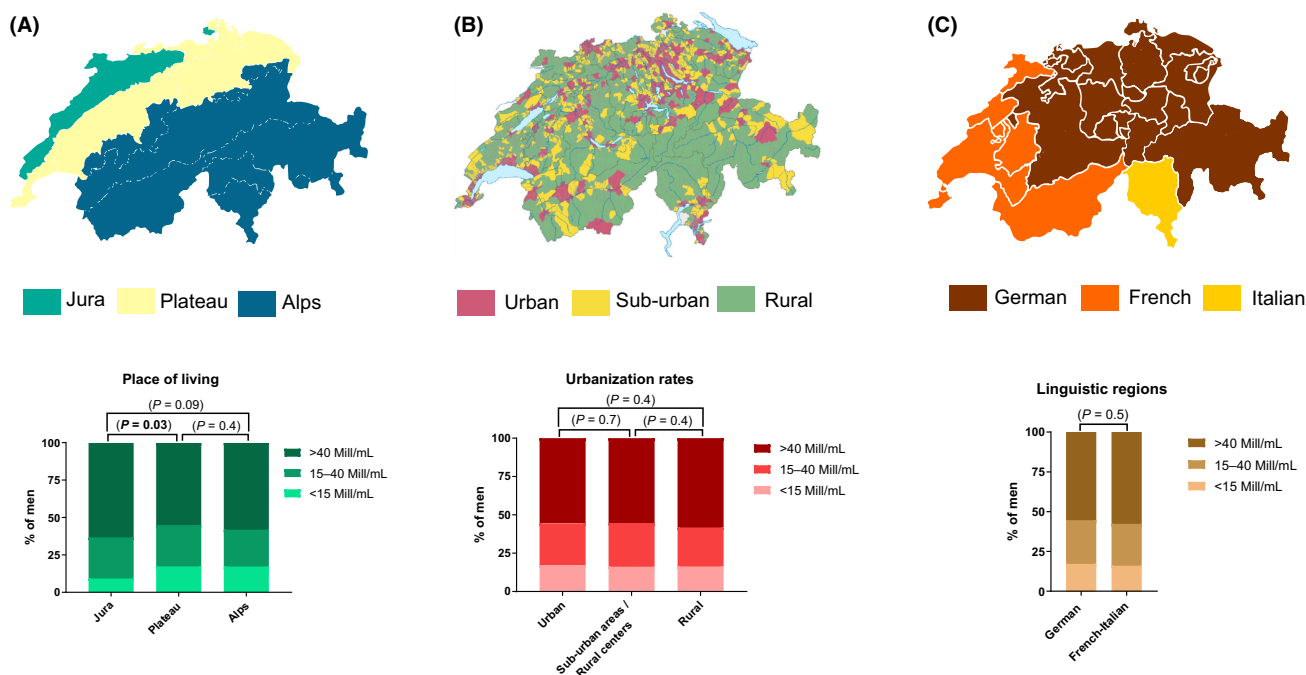
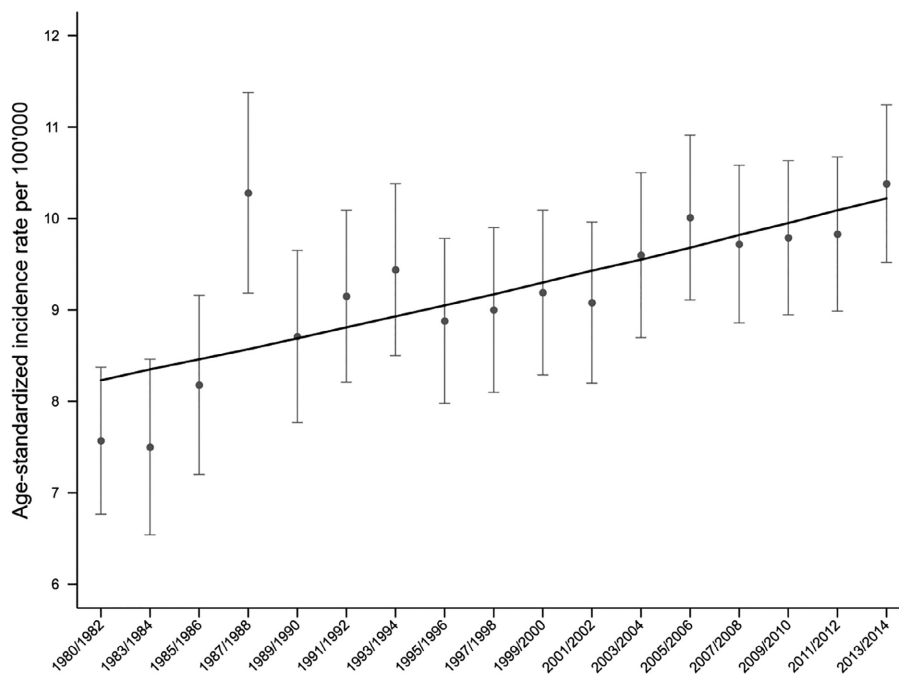


Figure 3 Temporal trend of testicular cancer incidence in Switzerland between 1980 and 2014. Gray dots and caps indicate the observed incidence rates with 95% confidence intervals, whereas the black line represents the modeled linear incidence trend. Over the 35-year period, incidence rates increase of 1.4% every two years (95% CI: 0.7–2.0; *p* < 0.001).



their reproductive capacities with only 0.1% having experienced fertility problems. The frequency of men diagnosed with varicocele during the physical examination in the study was of 18% compared with values ranging between 15 and 18% in the general Swiss population (Reinberg, 2007) pointing therefore to the

absence of selection bias in terms of fertility status or reproductive health. Evaluation of general traits such as BMI shows that values in our cohort (22.6 kg/m²) correspond almost exactly to what has been reported in a population of Swiss conscript (22.8 kg/m²) (Staub *et al.*, 2010) and to what have been reported

by the Swiss Office of Federal Statistics OFS (21.5 kg/m²) (OFS, 2018a). Additionally, the percentage of men having an educational level higher than obligatory school (86%) was very similar to the one in the general population (88%) as reported by the OFS (OFS, 2018b). We, thus, think that volunteers that participated in our study are also not self-selected in terms of BMI and education. It might also be argued that this group of young men does not represent the adult population as a whole. However, it has been reported that full sperm capacity is on average achieved by the age of 20 (Perheentupa *et al.*, 2016). As many as 86% were fully masculinized, having normal adult pubic hair—at least Tanner stage 5 (Garn, 1956)—and a mean testicular volume within the normal range (17.5 mL) (Bahk *et al.*, 2010). Thus, the immaturity of the volunteers could not explain the observed values.

According to a small survey, we conducted on the men who did not participate in the biological part of the study; the low participation rate can be partially explained by practical aspects such as difficulties in reaching the andrology laboratories from the army recruitment centers because of poor public transport connections, and the disappointment of some subjects following the military decision about their enrollment. Some men also mentioned being uncomfortable in donating a semen sample and having to undergo a genital examination or not being psychologically ready to learn the results.

Epidemiologically, the Testicular Dysgenesis Syndrome (TDS) hypothesis is corroborated by observations of generally lower semen quality among men from countries with high incidences of testicular cancer (Skakkebaek *et al.*, 2001). Indeed, a large set of countries within Europe including Germany, Denmark, and Norway reported both low-median sperm concentration and high testicular cancer rates. In countries such as Finland, Estonia, Lithuania, Latvia, and Spain, higher-median sperm concentration was observed with lower testicular cancer rates (Serrano *et al.*, 2013; Skakkebaek *et al.*, 2016). Consistently, we also observed a high incidence of testicular cancer along with an adjusted median sperm concentration that ranges among the lowest in Europe (Jørgensen *et al.*, 2011). The frequency of genital malformation such as cryptorchidism is not well reported in Switzerland, but comparison of the values we observed in our cohort (3.8%) with European and international reports reveals no differences (2.5–6%) (Boisen *et al.*, 2004; Ly *et al.*, 2008; Hart *et al.*, 2015).

A higher proportion of men with low sperm concentration was found to have been exposed to *in utero* maternal smoking and had suffered from previously diagnosed cryptorchidism and varicocele, in accordance with formerly published data (Storgaard *et al.*, 2003; Jensen *et al.*, 2005; Ramlau-Hansen *et al.*, 2007; Ravnborg *et al.*, 2011; Damsgaard *et al.*, 2016). Excluding those men did not change significantly the median sperm concentration, therefore indicating that impaired semen quality is not only because of the presence of those men in the study population. More importantly, this shows that, besides those previously described, many factors impacting semen quality remain unknown.

Compared with other European studies performed in urban places, one of the strengths of the present study is that reproductive health of young men was evaluated at a national level, with participants coming from every geographical region of the country. Because of the method used to sample each region

proportionally to the density of its residing male population, some regions had a low number of subjects. This made it difficult to compare data between regions and therefore hindered geographical analysis. We still attempted to identify possible factors that might affect semen quality either directly or indirectly. Differences according to the geographic region, urbanization rates, and linguistic regions were investigated. Our analysis revealed only subtle geographic variations with significant differences between Jura and Plateau among the three sperm concentration categories. We thus believe that more refined studies should be applied to improve our understanding of the influence of geographical factors during fetal and adult life on semen quality.

In conclusion, a significant proportion of Swiss young men display suboptimal semen quality, with only 38% of the men having sperm concentration, motility, and morphology values that are above the WHO reference values. A consequence will likely be prolonged waiting time to pregnancy and an increased risk of seeking fertility treatment.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Béatrice Conne, Dr. Roumen Parapanov, and Cadia Cataldo for the sperm morphology analysis, to Prof. Jaime Mendiola for statistical assistance, and to Dr. Friedrich von Toggenburg, Michel Crausaz, and Dr. Christoph Zeller for data and sample collection. Authors are appreciative to Pr. Hy Dao from the Department of Geography and Environment of the University of Geneva for providing the data on urbanization in Switzerland and Dr. Luc Henry for proofreading and useful comments during the manuscript preparation. Authors are also thankful to Prof. Felix Althaus—President of NFP-50 Program—SNSF, Dr. Gianpiero LUPI—Former Head physician of the Swiss army and to the directions of Fertasa SA and MCL SA. The technicians are very appreciated for the data collection and analysis, the physicians for their urogenital assessments, and all the young men who participated in the study. This work was supported by the Swiss Centre for Applied Human Toxicology—SCAHT and the ‘Fondation privée des Hôpitaux Universitaires de Genève’. The collection of human biological material used for this study was supported by the Swiss National Science Foundation (SNSF)—NFP 50 ‘Endocrine Disruptors: Relevance to Humans, Animals and Ecosystems’, the Medical Services of Swiss Army (DDPS), the FABER Foundation and Medisupport. The Swiss cancer data used in these analyses were supplied by the Foundation National Institute for Cancer Epidemiology and Registration (NICER) and its partner registries in cantons (alphabetical order) Geneva, Glarus & Graubünden, Neuchâtel & Jura, St. Gallen & Appenzell-Innerrhoden & Appenzell-Ausserrhoden, Ticino, Valais, Vaud, Zuerich & Zug. Members of the NICER Working Group for these analyses include (alphabetical order of cantons): Geneva — C. Bouchardy; Glarus & Graubünden — C. Herrmann, M. Mousavi; Neuchâtel & Jura — J.-L. Bulliard, M. Maspoli; St. Gallen & Appenzell; Ticino — A. Bordoni; Valais — I. Konzelmann; Vaud — R. Blanc-Moya; Zuerich & Zug — S. Rohrmann.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR'S CONTRIBUTIONS

R.R., A.S., E.S., M.G., and S.N. designed the study. R.R., A.S., E.S. M.V.dB., J.V., A.F. collected the data. R.R., L.P., A.S., R.G., L.M., T.K.J., N.E.S., N.J., and S.N. analyzed and interpreted the data. R.R., A.S., N.J., and S.N. wrote the paper. All authors were involved in paper revision and gave final approval.

REFERENCES

- Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson AM, Krarup T, Keiding N & Skakkebaek NE. (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Hum Reprod* 15, 366–372.
- Bahk JY, Jung JH, Jin LM & Min SK. (2010) Cut-off value of testes volume in young adults and correlation among testes volume, body mass index, hormonal level, and seminal profiles. *Urology* 75, 1318–1323.
- Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto A-M, Schmidt IM, Chellakooty M, Damgaard IN, Mau C, Reunanen M, *et al.* (2004) Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet (London, England)* 363, 1264–1269.
- Bonde JPE, Ernst E, Jensen TK, Hjollund NHI, Kolstad H, Scheike T, Giwercman A, Skakkebaek NE, Henriksen TB & Olsen J. (1998) Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 352, 1172–1177.
- Carlsen E, Giwercman A, Keiding N & Skakkebaek NE. (1992) Evidence for decreasing quality of semen during past 50 years. *BMJ* 305, 609–613.
- Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, *et al.* (2010) World Health Organization reference values for human semen characteristics*. *Hum Reprod Update* 16, 231–245.
- Damsgaard J, Joensen UN, Carlsen E, Erenpreiss J, Blomberg Jensen M, Matulevicius V, Zilaitiene B, Olesen IA, Perheentupa A, Punab M, *et al.* (2016) Platinum Priority – Andrology Varicocele Is Associated with Impaired Semen Quality and Reproductive Hormone Levels: A Study of 7035 Healthy Young Men from Six European Countries, 0–1.
- Deonandan R & Jaleel M. (2012) Global decline in semen quality: ignoring the developing world introduces selection bias. *Int J Gen Med* 5, 303–306.
- Dubin L & Amelar RD. (1970) Varicocele size and results of varicolectomy in selected subfertile men with varicocele. *Fertil Steril* 21, 606–609.
- Erenpreiss J, Punab M, Zilaitiene B, Hlevicka S, Zayakin P, Matulevicius V, Tomas Preiksa R & Jørgensen N. (2017) Semen quality of young men from the general population in Baltic countries. *Hum Reprod* 32, 1334–1340.
- Fernandez MF, Duran I, Olea N, Avivar C, Vierula M, Toppari J, Skakkebaek NE & Jørgensen N. (2012) Semen quality and reproductive hormone levels in men from Southern Spain. *Int J Androl* 35, 1–10.
- Garn SM (1956) Growth at adolescence. By J. M. Tanner. Pp. vii + 212. Blackwell Scientific Publications, Oxford. Publisher simultaneously by Charles C Thomas and the Ryerson Press. 1955. *Am J Phys Anthropol* 14, 120–122.
- Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, *et al.* (2001) Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med* 345, 1388–1393.
- Hart RJ, Doherty DA, McLachlan RI, Walls ML, Keelan JA, Dickinson JE, Skakkebaek NE, Norman RJ & Handelsman DJ. (2015) Testicular function in a birth cohort of young men. *Hum Reprod* 30, dev244.
- Hauser R, Godfrey-Bailey L & Chen Z. (2005) Does the potential for selection bias in semen quality studies depend on study design? Experience from a study conducted within an infertility clinic. *Hum Reprod* 20, 2579–2583.
- Iwamoto T, Nozawa S, Mieno MN, Yamakawa K, Baba K, Yoshiike M, Namiki M, Koh E, Kanaya J, Okuyama A, *et al.* (2013) Semen quality of 1559 young men from four cities in Japan: a cross-sectional population-based study. *BMJ Open* 3, e002222.
- Jensen MS, Mabeck LM, Toft G, Thulstrup AM & Bonde JP. (2005) Lower sperm counts following prenatal tobacco exposure. *Hum Reprod* 20, 2559–2566.
- Jørgensen N. (2001) Regional differences in semen quality in Europe. *Hum Reprod* 16, 1012–1019.
- Jørgensen N. (2002) East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. *Hum Reprod* 17, 2199–2208.
- Jørgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, Virtanen HE, Skakkebaek NE & Toppari J. (2011) Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *Int J Androl* 34, e37–e48.
- Kim HH & Goldstein M. (2008) A new clinical grading system for varicocele. *Fertil Steril* 90, S467.
- Le Moal J, Rolland M, Gorla S, Wagner V, De Crouy-Chanel P, Rigou A, De Mouzon J & Royère D. (2014) Semen quality trends in French regions are consistent with a global change in environmental exposure. *Reproduction* 147, 567–574.
- Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R & Swan SH. (2017) Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum Reprod Update* 23, 646–659.
- Lopez M, Bordoni A, Bouchardy C, Bulliard JL, Camey B, Dehler S, Frick H, Konzelmann I, Maspoli M, Mousavi SM, *et al.* (2017) Evaluation of completeness of case ascertainment in Swiss cancer registration. *Eur J Cancer Prev* 26, S139–S146.
- Ly WK, Gapany C, Frey P, Cachat F, Gudinchet F, Jichlinski P, Meyrat B-J, Ramseyer P, Theintz G & Burnand B. (2008) Management of cryptorchidism in children: guidelines. Available from: <http://swisspu.ch/pdfempfehlungen/7.pdf>.
- Mendiola J, Stahlhut RW, Jørgensen N, Liu F & Swan SH. (2011) Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. *Environ Health Perspect* 119, 958–963.
- Mendiola J, Jørgensen N, Mínguez-Alarcón L, Sarabia-Cos L, López-Espín JJ, Vivero-Salmerón G, Ruiz-Ruiz KJ, Fernández MF, Olea N, Swan SH, *et al.* (2013) Sperm counts may have declined in young university students in Southern Spain. *Andrology* 1, 408–413.
- Menkveld R, Stander FS, Kotze TJ, Kruger TF, & van Zyl JA. (1990) The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. *Hum Reprod* 5, 586–592.
- Muller A, La Rochebrochard E, De Labbé-Declèves C, Jouannet P, Bujan L, Mieusset R, Le Lannou D, Guerin JF, Benchaib M, Slama R, *et al.* (2004) Selection bias in semen studies due to self-selection of volunteers. *Hum Reprod* 19, 2838–2844.
- OFS. Pratiques linguistiques en Suisse - Premiers résultats de l'Enquête sur la langue, la religion et la culture 2014 | Publication; 2016. Available from: <https://www.bfs.admin.ch/bfs/en/home/statistics/catalogues-databases/publications.assetdetail.1000171.html>
- OFS. Indice de masse corporelle (IMC) par sexe, âge, niveau de formation, région linguistique - 1992, 1997, 2002, 2007, 2012, 2017 | Tableau; 2018a. Available from: <https://www.bfs.admin.ch/bfs/fr/home/statistiques/sante/determinants/exces-poids.assetdetail.6466024.html>
- OFS. Niveau de formation de la population résidante permanente selon l'âge et le sexe - 1999-2017 | Tableau; 2018b. Available from: <https://www.bfs.admin.ch/bfs/fr/home/statistiques/education-science/niveau-formation-competences.assetdetail.4802235.html>
- Paasch U, Salzbrunn A, Glander HJ, Plambeck K, Salzbrunn H, Grunewald S, Stucke J, Skakkebaek NE & Jørgensen N. (2008) Semen quality in sub-fertile range for a significant proportion of young men from the general German population: a co-ordinated,

- controlled study of 791 men from Hamburg and Leipzig. *Int J Androl* 31, 93–102.
- Palermo G, Joris H, Devroey P & Van Steirteghem AC. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 340, 17–18.
- Perheentupa A, Sadov S, Rönkä R, Virtanen HE, Rodprasert W, Vierula M, Jørgensen N, Skakkebaek NE & Toppari J. (2016) Semen quality improves marginally during young adulthood: a longitudinal follow-up study. *Hum Reprod* 31, 502–510.
- Priskorn L, Nordkap L, Bang AK, Krause M, Holmboe SA, Egeberg Palme DL, Winge SB, Mørup N, Carlsen E, Joensen UN, *et al.* (2018) Average sperm count remains unchanged despite reduction in maternal smoking: results from a large cross-sectional study with annual investigations over 21 years. *Hum Reprod* 33, 998–1008.
- Punab M, Zilaitiene B, Jørgensen N, Horte A, Matulevicius V, Peetsalu A & Skakkebaek NE. (2002) Regional differences in semen qualities in the Baltic region. *Int J Androl* 25, 243–252.
- Ramlau-Hansen CH, Thulstrup AM, Storgaard L, Toft G, Olsen J & Bonde JP. (2007) Is prenatal exposure to tobacco smoking a cause of poor semen quality? A follow-up study *Am J Epidemiol* 165, 1372–1379.
- Ravnborg TL, Jensen TK, Andersson A-M, Toppari J, Skakkebaek NE & Jørgensen N. (2011) Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index. *Hum Reprod* 26, 1000–1011.
- Reinberg O. Varicocèle de l'enfant et de l'adolescent – Revue Médicale Suisse; 2007. Available from: <https://www.revmed.ch/RMS/2007/RMS-136/32748>
- Richthoff J, Rylander L, Hagmar L, Malm J & Giwercman A. (2002) Higher sperm counts in Southern Sweden compared with Denmark. *Hum Reprod* 17, 2468–2473.
- Rodprasert W, Virtanen HE, Sadov S, Perheentupa A, Skakkebaek NE, Jørgensen N & Toppari J. (2019) An update on semen quality among young Finnish men and comparison with Danish data. *Andrology* 7, 15–23.
- Sakamoto H, Saito K, Ogawa Y & Yoshida H. (2007) Testicular volume measurements using prader orchidometer versus ultrasonography in patients with infertility. *Urology* 69, 158–162.
- al Salim A, Murchison PJ, Rana A, Elton RA & Hargreave TB. (1995) Evaluation of testicular volume by three orchidometers compared with ultrasonographic measurements. *Br J Urol* 76, 632–635.
- Serrano T, Chevrier C, Multigner L, Cordier S & Jegou B. (2013) International geographic correlation study of the prevalence of disorders of male reproductive health. *Hum Reprod* 28, 1974–1986.
- Skakkebaek NE, Rajpert-De Meyts E & Main KM. (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 16, 972–978.
- Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson A-M, Eisenberg ML, Jensen TK, Jørgensen N, Swan SH, Sapra KJ, *et al.* (2016) Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiol Rev* 96, 55–97.
- Slama R, Eustache F, Ducot B, Jensen TK, Jørgensen N, Horte A, Irvine S, Suominen J, Andersen AG, Auger J, *et al.* (2002) Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Hum Reprod* 17, 503–515.
- Staub K, Rühli FJ, Woitek U & Pfister C. (2010) BMI distribution/social stratification in Swiss conscripts from 1875 to present. *Eur J Clin Nutr* 64, 335–340.
- Storgaard L, Bonde JP, Ernst E, Spanò M, Andersen CY, Frydenberg M & Olsen J. (2003) Does smoking during pregnancy affect sons' sperm counts? *Epidemiology* 14, 278–286.
- Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, Hatch M, Redmon JB, Wang C, Overstreet JW & Study For Future Families Research Group SFFFR (2003) Geographic differences in semen quality of fertile U.S. males. *Environ Health Perspect* 111, 414–420.
- Tsarev I, Gagonin V, Giwercman A & Erenpreiss J. (2005) Sperm concentration in Latvian military conscripts as compared with other countries in the Nordic-Baltic area. *Int J Androl* 28, 208–214.
- Viktor Goebel FK. Areas with urban character 2012; 2014. Available at: <https://www.bfs.admin.ch/bfsstatic/dam/assets/349566/master>.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Locations of the Swiss army recruitment centers. Name of cantons are indicated in red and name of centers in black.

Figure S2. Number of volunteers contacted and the participation rate according to their type of participation in the study.

Table S1. Description of Swiss men according to geographical stratification in three regions.

Table S2. Description of Swiss men according to urbanization rates.

Table S3. Description of Swiss men according to linguistic regions.