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# Alcohol intake and semen variables: cross-sectional analysis of a prospective cohort study of men referring to an Italian Fertility Clinic

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## SUMMARY

***Background:*** The association between alcohol intake and male reproductive function is still controversial. In the frame of a prospective cohort study, designed to investigate the relation between life style and fertility, we performed a cross-sectional analysis of semen quality.

***Methods:*** Men of subfertile couples, referring to an Italian Infertility Unit and eligible for assisted reproductive techniques (ARTs), were asked about their lifestyle: BMI, smoking, caffeine intake, occupational and leisure physical activity (PA) and alcohol intake in the last year before ART procedure. Semen volume, sperm concentration, total sperm count and sperm motility were determined. Age, risk factors for impaired male fertility, caffeine, smoking, leisure PA, days of abstinence and daily calories intake were accounted for in the analyses.

***Results:*** Between September 2014 and December 2016, we enrolled 323 male patients, mean age 39.3 years. Thirty-one (9.6%) were abstainers, 97 (30.0%) drank <1–3, 98 (30.3%) 4–7 and 97 (30.0%) ≥8 alcohol units per week. As compared to men drinking <1–3 units per week, median semen volume was higher in the 4–7 units/week group (3.0 mL, interquartile range, IQR, 2.0–4.0 vs. 2.4 mL, IQR 1.7–3.5), as well as total sperm count (87.9 mil/mL, IQR 20.2–182.1 vs. 51.5 mil/mL, IQR 15.2–114.7). Association with sperm concentration was also significant, with a U-shaped trend in groups of alcohol intake. After adjusting for potential confounders, these relations were confirmed. Similar patterns were observed in subgroups of leisure PA and risk factors for impaired male fertility, although these estimates often lacked statistical significance, presumably because of low sample size.

***Conclusions:*** Moderate alcohol intake appears positively associated to semen quality in male partners of infertile couples undergoing ARTs.

## INTRODUCTION

Approximately 15% of couples in their reproductive age is affected by fertility problems and male factors seem to account for up to 30% of cases (Nyboe Andersen *et al.*, 2008; Thoma *et al.*, 2013). A comprehensive, evidence-based meta-analysis has recently shown an overall 32% decline in sperm concentration in European men over the past 50 years (Sengupta *et al.*, 2017).

In most cases, the suboptimal semen quality is of idiopathic origin, with no clear explanation for impaired spermatogenesis. Although the causal link between environmental factors and impaired male fertility is still weak, there is evidence suggesting that semen quality may be influenced by environmental conditions and lifestyle habits (Gabrielsen & Tanrikut, 2016); among others, and besides the well-known genetic and endocrine factors (Visser & Repping, 2010; Ohlander *et al.*, 2016), smoking, overweight, physical activity, dietary factors and alcohol intake have been suggested to play a role (Mendiola *et al.*, 2009; Li *et al.*, 2011; Afeiche *et al.*, 2013). However, evidence is not always consistent.

A negative association between alcohol intake and semen quality has been suggested by some authors (Martini *et al.*, 2004; Muthusami & Chinnaswamy, 2005), although other studies did not confirm this finding (López Teijón *et al.*, 2007; Hansen *et al.*, 2012). According to a recent meta-analysis of 15 cross-sectional studies, occasional consumption does not adversely affect semen variables, whereas a negative association with semen

volume and normal morphology emerged for daily consumption (Ricci *et al.*, 2017). However, these findings could not be controlled for confounders such as smoking and age.

To provide further information on this topic, we analysed data from a study on the impact of lifestyle habits and diet on Assisted Reproductive Techniques (ARTs) in Italian infertile couples focusing on the relation between alcohol intake and semen variables.

## MATERIALS AND METHODS

From September 2014 to December 2016, in randomly selected days, subfertile couples, presenting for evaluation to the Infertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore, Policlinico, Milan, and eligible for ART, were invited to participate in an ongoing prospective cohort study on the role of lifestyle habits and diet on ART outcome. The study protocol was approved by the local Institutional Review Board. All procedures were in accord with the Helsinki Declaration and all participants provided written informed consent.

Study participation was proposed during the diagnostic phase. Couples were interviewed on the day of oocyte retrieval. On the same day, a semen sample was collected and analysed prior to proceeding with in vitro fertilization (IVF) or intra-cytoplasmic sperm injection (ICSI). The time interval between the proposal of the study and the interview was generally less than one month.

Both partners of couples who agreed to participate were interviewed by centrally trained personnel, using a standard questionnaire to obtain information on general socio-demographic characteristics, personal and health history and habits (including smoking, physical activity, alcohol intake and methylxanthine-containing beverages consumption). Couples who do not speak fluent Italian were excluded.

The present study reported exclusively on evidence obtained from the male partner.

The overall participation rate was close to 95%. This high participation rate was mainly due to the fact that couples were interviewed during the period spent waiting for the different diagnostic and therapeutic phases. Considering this time off and the non-sensitive character questions, couples did not generally refuse to participate.

Information on diet was obtained using a previously validated food frequency questionnaire (FFQ) (Franceschi *et al.*, 1993, 1995; Decarli *et al.*, 1996). Patients were asked to report about their usual weekly food consumption in the last year. The FFQ includes the average weekly consumption of 78 food items or food groups (such as the major sources of animal fats – i.e. red meat, milk, cheese, ham, salami – folates, vitamins – vegetables and fruit – pasta and bread consumption, cake, sweets and chocolate, fish) and beverages. Intakes lower than once per week, but at least once per month, were coded 0.5 per week. Seasonal consumption was also considered (week consumption of vegetables/fruits available in limited periods during the year, weighted for months of consumption). Energy and mineral, macro- and micronutrient intake was estimated using the most recent update of an Italian food consumption database (Gnagnarella *et al.*, 2004).

Body mass index (BMI) was classified according to World Health Organization (WHO) indications (WHO, Health Topics, BMI http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle/body-mass-index-bmi).

Men were considered as having risk factors for impaired fertility, if they had a history of previous chemio- or radiotherapy, as well as previous reproductive organ diseases, such as orchiectomy, cryptorchidism and varicocoele. These data were retrieved from clinical records.

Smoking habits were categorized as never, former or current, and number of cigarettes smoked daily and duration of smoking were recorded.

Caffeine intake from coffee (60 mg per cup), cappuccino (75 mg per cup), tea (45 per cup), decaffeinated coffee (4 per cup) and chocolate (6 mg/10 g) was calculated (Tavani, 2013).

Occupational physical activity (PA) was described as heavy (or very heavy), light/moderate, mainly standing or mainly sitting. Leisure PA was recorded in term of hours/week: <2, 2 to 4, ≥5. No information was collected about intensity or type of leisure PA.

Calories intake was calculated by the FFQ (Franceschi *et al.*, 1993, 1995; Decarli *et al.*, 1996).

Information on alcohol intake was collected as usual weekly consumption (1 unit = 125 mL wine or 330 mL beer or 30 mL spirits, all containing approximately 12.5 g of ethanol). An intake lower than one unit per week was codified as 0.5.

### Sperm analysis

Men were instructed to abstain from ejaculation for 2–5 days before semen analysis and to report the specific time of abstinence. Semen samples were obtained by masturbation and collected into a sterile plastic container provided and labeled with the date and time of collection. All seminal fluid examinations were carried out by the laboratory of the Unit, where samples were maintained at room temperature until complete liquefaction. Duration of complete liquefaction (<1 h) was documented, until 1 h was reached. Semen analysis was performed with standardized methods according to the WHO guidelines (World Health Organization 2010). The following variables were taken into consideration: volume (mL), sperm concentration (spermatozoa N/mL) and motility (%). Sperm motility was classified into total (progressive + non-progressive motility) and progressive. Total sperm count was calculated as volume × sperm concentration. As semen samples were collected specifically to carry out ART procedures, sperm morphology was evaluated only in partners of those couples undergoing IVF and after semen capacitation (and not on rough samples).

The laboratory personnel was trained using the ESHRE Special Interest Group in Andrology Basic Semen Analysis Course (Barratt *et al.*, 2011).

### Statistical procedures

Categorical or ordinal variables were described as frequency (percentage,%), continuous variables as mean (standard deviation, SD) if normally distributed and medians (interquartile range, IQR) if not. Four domains of semen quality were assessed: volume, concentration, total count and motility. At the univariate analysis, groups were compared by means of Kruskal–Wallis test, even if they were normally distributed.

In order to perform a multivariate analysis including potential confounders, non-normal (skewed) distributions of semen parameters were square-root transformed and included in a general linear model. Adjusted medians and 95% confidence interval (CI) were calculated back-transforming the adjusted means and their 95% CIs. In the model, we included as potential

 

confounders variables associated to alcohol intake or semen quality at univariate analysis. Given that the relation between alcohol intake and sperm parameters was potentially different in men with or without risk factors for impaired fertility, we planned to perform an analysis in strata for this variable. The multivariate analysis also showed a significant relation between sperm motility and leisure PA, therefore we also performed a further analysis in strata for PA.

All reported *p*-values are based on two-sided tests and considered statistically significant if below 5%.

## RESULTS

From September 2014 to December 2016, 327 men were enrolled, aged 39.2 years on average (SD 5.2, range 27–60). Among them, four did not provide complete information about lifestyle and were excluded. The final analyses were conducted in a sample of 323 men, aged 39.3 years on average (SD 5.3, range 27–60).

The median daily alcohol intake was 8.30 g (IQR 2.72–15.95). Excluding 31 men who did not drink at all, we determined tertiles of daily alcohol intake: 0.01–5.44, 5.45–14.20 and ≥14.21 g per day. Tertiles corresponded to a weekly consumption of <1–3, 4–7 and ≥8 alcohol units, respectively. In the last category, the highest value of consumption was 108.13 g/day (60 units per week), while the median alcohol consumption was 21.21 g/day (about 12 units per week).

Patients' characteristics according to alcohol intake are described in Table 1: alcohol intake was inversely associated with age, and positively with caffeine consumption and calories intake, although the highest caloric intake was observed in abstainers. Never smokers were less frequently alcohol drinkers than both former and current smokers.

Table 2 shows the median values of semen variables according to demographic characteristics and lifestyle patterns. Alcohol intake was associated to semen volume, sperm concentration and total sperm count, with no dose-effect relation. Men drinking 4–7 alcohol units per week had the highest semen volume. The highest median concentrations were observed in abstainers and in men drinking ≥8 units/week; total count was also associated to alcohol intake, but did not show a dose-dependent relation, although a significant rank correlation was observed between these two variables (Spearman rho = 0.12, $p = 0.038$).

Days of abstinence were positively correlated to semen volume (Spearman rho = 0.14, $p = 0.01$) and inversely to sperm motility (Spearman rho = −0.11), with borderline significance ($p = 0.07$).

We accounted for the observed difference among men in groups of alcohol intake using a general linear model equation, that included age (associated to alcohol intake and semen variables), days of abstinence, leisure PA, risk factors for impaired male fertility (associated with at least one semen variable), smoking status, caffeine consumption, calories intake (associated with alcohol intake). Previous ART cycles did not relate to alcohol intake nor to semen quality: therefore this variable was not included in the final model. However, we also reran the model including this information, without significant modifications in the results.

In the multivariate analysis, we still found a relation between alcohol intake and semen volume, concentration and total count (Table 3). Back-transforming semen volume, and using men with <1–3 units per week of alcohol intake as the reference group, we observed that men drinking 4–7 units/week had a significantly higher median semen volume, that both men in 4–7 and ≥8 units/week group had significantly higher sperm concentration ($p = 0.047$ and $p = 0.004$, respectively) and that abstainers had higher median concentration as well ($p = 0.017$). Total count was also associated to alcohol intake: men drinking 4–7 and ≥8 units/week had higher total count than men drinking <1–3 units/week ($p = 0.006$ and $p = 0.009$, respectively) but without dose-dependent relation. No association emerged with sperm motility.

In the multivariate model, the presence of risk factors for impaired male fertility was significantly associated to worse sperm concentration (19.4 vs. 40.9 mil/mL, $p < 0.0001$), total count (48.8 vs. 100.2 mil, $p = 0.0002$) and motility (29.3% vs. 39.6%, $p < 0.0001$). Leisure PA was related to sperm motility, with the lowest motility in the intermediate level of PA: 31.3% in men with 2–4 h per week vs. 37.0% in those with ≥5 h per week ($p = 0.012$) of leisure PA.

A further analysis was performed, aiming at better understanding the role of alcohol intake in strata of impaired male fertility and physical activity: medians and 95% CIs of sperm variables are shown in Table 3, according to alcohol intake. Considering an alcohol intake of <1–3 units/week as the reference category, we found that semen volume was significantly lower in abstainers with low level of leisure PA; a trend of increasing volume with increasing alcohol intake, with a maximum at 4–7 units/week was consistently found in all strata.

Concentration and total sperm count increased with higher level of alcohol intake in men without risk factors for impaired fertility, and was significant both in those drinking 4–7 and ≥8 units per week. As regards leisure PA, no significant relation was observed in men with ≥5 h/week, whereas in subject with <2 and 2–4 h/week concentration and total count were positively related to alcohol intake, although no dose-relation was seen. As in the overall analysis, alcohol intake was not associated with sperm motility in any subgroups.

We checked terms for interactions between alcohol and, in turn, smoking, PA, risk factors for male impaired fertility, age class and caffeine intake. None of them was significant (data not shown).

Lastly, we estimated the association between high alcohol intake and semen quality, comparing 39 men who drank ≥14 units/week: in a model including the aforementioned variables, no statistically or clinically significant association was observed, both including and excluding non-drinkers from the analysis.

## DISCUSSION

In this study, moderate alcohol intake appeared associated with increased semen volume, sperm concentration and total sperm count in the whole sample. A similar pattern was observed in subgroups of leisure PA and risk factors for impaired male fertility, although these estimates often lacked statistical significance.

Considering that in our study both semen volume and sperm concentration were positively associated to alcohol consumption, total sperm count was positively related to alcohol intake as well.

In our analysis, moderate alcohol intake relation with sperm concentration and total count was significant in the entire cohort, in men without risk factors for impaired fertility and in

**Table 1** Demographic characteristics and lifestyle patterns according to tertiles of alcohol intake

| | Overall *N* = 323 (%) | Alcohol intake (units/week) | | | | *p*[a] |
|---|---|---|---|---|---|---|
| | | Abstainers *N* = 31 (9.6%) | <1–3 *N* = 97 (30.0%) | 4–7 *N* = 98 (30.3%) | ≥8 *N* = 97 (30.0%) | |
| Alcohol intake (g, median, range) | 8.3 (0–108.13) | 0 (0–0) | 2.63 (0.85–5.44) | 9.58 (5.70–14.20) | 21.21 (14.27–108.13) | - |
| Age | | | | | | |
| <35 | 60 (18.6) | 3 (9.7) | 13 (13.4) | 19 (19.4) | 25 (25.8) | |
| 35–39 | 126 (39.0) | 7 (22.6) | 39 (40.2) | 42 (42.9) | 38 (39.2) | |
| ≥40 | 137 (42.4) | 21 (67.7) | 45 (46.4) | 37 (37.8) | 34 (35.0) | 0.0007 |
| Mean ± SD | 39.3 ± 5.2 | 41.4 ± 5.0 | 40.1 ± 5.4 | 38.8 ± 4.8 | 38.4 ± 5.4 | 0.01[b] |
| College degree | 131 (40.6) | 9 (29.0) | 40 (41.2) | 44 (44.9) | 38 (39.2) | 0.55 |
| Risk factor for impaired male fertility | 66 (20.4) | 3 (9.7) | 20 (20.6) | 22 (22.4) | 21 (21.6) | 0.28 |
| Previous ART cycles | 184 (57.0) | 19 (61.3) | 54 (55.7) | 57 (58.2) | 54 (55.7) | 0.75 |
| Days of abstinence mean ± SD | 3.9 ± 1.9 | 4.0 ± 2.5 | 3.9 ± 1.9 | 4.0 ± 2.2 | 3.8 ± 1.5 | 0.85[b] |
| BMI | | | | | | |
| <25.0 | 146 (45.3) | 16 (51.6) | 41 (42.3) | 54 (55.1) | 35 (36.5) | |
| 25.0–29.9 | 148 (46.0) | 13 (41.9) | 46 (47.4) | 33 (33.7) | 56 (58.3) | |
| ≥30.0 | 28 (8.7) | 2 (6.4) | 10 (10.3) | 11 (11.2) | 5 (5.2) | 0.54 |
| Mean ± SD | 25.3 ± 3.0 | 24.6 ± 2.8 | 25.5 ± 3.1 | 25.0 ± 3.2 | 25.5 ± 2.8 | 0.25[b] |
| Smoking | | | | | | |
| Never | 129 (39.4) | 17 (54.8) | 50 (51.6) | 37 (37.8) | 25 (25.8) | |
| Former | 93 (28.9) | 6 (19.4) | 22 (22.7) | 32 (32.6) | 33 (34.0) | |
| Current | 101 (31.7) | 8 (25.8) | 25 (25.8) | 29 (29.6) | 39 (40.2) | 0.004 |
| 0–9 cig/day | 46 (14.1) | 2 (6.4) | 13 (13.4) | 18 (18.4) | 14 (14.4) | |
| ≥10 cig/day | 15 (13.9) | 6 (19.4) | 12 (12.4) | 11 (11.2) | 25 (25.8) | 0.001 |
| Caffeine intake (mg/day) | | | | | | |
| 0–127 | 110 (34.1) | 15 (48.4) | 38 (39.2) | 30 (30.6) | 27 (27.8) | |
| 128–214 | 105 (32.5) | 9 (29.0) | 29 (29.9) | 41 (41.8) | 26 (26.8) | |
| ≥215 | 108 (33.4) | 7 (22.6) | 30 (30.9) | 27 (27.6) | 44 (45.4) | 0.005 |
| Mean ± SD | 175 ± 99 | 152 ± 108 | 168 ± 100 | 177 ± 89 | 189 ± 105 | 0.24[b] |
| Occupational physical activity | | | | | | |
| Heavy | 67 (20.8) | 12 (38.7) | 17 (17.5) | 19 (19.4) | 19 (19.8) | |
| Light/moderate | 68 (21.1) | 9 (29.0) | 20 (20.6) | 21 (21.4) | 18 (18.8) | |
| Mainly standing | 47 (16.6) | 4 (12.9) | 17 (17.5) | 10 (10.2) | 16 (16.7) | |
| Mainly sitting | 140 (43.5) | 6 (19.4) | 43 (44.3) | 48 (49.0) | 43 (44.8) | 0.07 |
| Leisure physical activity | | | | | | |
| <2 h/week | 133 (41.7) | 19 (63.3) | 47 (49.0) | 29 (29.6) | 38 (40.0) | |
| 2–4 h/week | 112 (35.1) | 6 (20.0) | 29 (30.2) | 41 (41.8) | 36 (37.9) | |
| ≥5 h/week | 74 (22.1) | 5 (16.7) | 20 (20.8) | 28 (28.6) | 21 (22.1) | 0.11 |
| Calories intake (kcal/day) median (IQR) | 1899 (1623–2290) | 2110 (1795–2377) | 1740 (1429–2145) | 1858 (1720–2257) | 2028 (1683–2404) | 0.0002[c] |

[a]Cochran-Mantel-Hanszel chi-square test, if not otherwise indicated. [b]Analysis of variance. [c]Kruskal–Wallis test.

those with low and intermediate level of leisure PA. Actually, this trend was observed in all subgroups considered in our analyses: in some cases, differences were not significant, probably because of the small sample size of each group.

A study on 1221 young Danish men (Jensen *et al.*, 2014) found that sperm concentration and total sperm count were negatively associated with increasing habitual alcohol intake. A case–control study (Muthusami & Chinnaswamy, 2005) concluded that semen volume and sperm concentration were lower in alcoholics compared with abstainers. However, in other studies alcohol did not seem to play any role. Considering the peculiar group of men enrolled from Fertility Clinics, Martini *et al.* (2004) and López Teijón *et al.* (2007) found no association, whereas Goverde *et al.* (1995) reported that alcohol did not seem to be associated with poor semen quality, although excessive alcohol consumption may affect an already suboptimal sperm morphology.

The inconsistency between our findings and previous studies may be due to the different way of categorization of alcohol consumption and to the different drinking habits of the populations studied. For example, in Martini *et al.*'s study (Martini *et al.*, 2004), the comparison was performed between patients who drank any quantity of alcohol and those who did not drink at all in the past six months, therefore the effect of low and high alcohol intake (about 25% of drinkers included in the study consumed more than 28 units/week) could not be discerned.

Men included in the study of Jensen *et al.* (2014) also had higher levels of alcohol intake than subjects in our sample: although the negative effects of alcohol intake were consistently found at high doses (in men who drank more than 25

 

**Table 2** Median sperm parameters (interquartile range) according to demographic characteristics and lifestyle patterns

| Characteristics | *N* | Volume (mL) | | Concentration (mil/mL) | | Total count (mil) | | Motility (A+B) % | |
|---|---|---|---|---|---|---|---|---|---|
| | | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 |
| Overall | 323 | 2.6 | 1.7–3.7 | 32 | 9.7–67.0 | 76 | 27.4–155.7 | 41 | 30.0–49.0 |
| Age | | | | | | | | | |
| <35 | 60 | **2.8** | **2.0–3.5** | 45 | 15.9–72.0 | **116.8** | **35.7–213.0** | 44 | 31.0–54.0 |
| 35–39 | 126 | **2.7** | **1.8–4.0** | 30 | 8.7–57.0 | **70** | **21.4–144.0** | 40 | 29.0–50.0 |
| ≥40 | 137 | **2.3** | **1.4–3.5** | 30 | 10.0–70.0 | **75.4** | **21.6–138.0** | 40 | 31.0–46.0 |
| College degree | | | | | | | | | |
| No | 192 | 2.6 | 1.8–3.7 | 30.5 | 9.7–68.0 | 75.2 | 29.9–156.0 | 42 | 31.0–50.0 |
| Yes | 131 | 2.7 | 1.5–3.7 | 32 | 9.8–57.0 | 78.9 | 24.0–144.0 | 40 | 28.0–48.0 |
| Risk factor for impaired male fertility | | | | | | | | | |
| No | 257 | 2.5 | 1.5–3.5 | **37** | **13.7–72.0** | **88.8** | **34.5–166.5** | **42** | **32.5–50.0** |
| Yes | 66 | 2.9 | 1.9–4.5 | **13.7** | **4.7–34.0** | **36.2** | **15.2–72.0** | **33** | **21.0–45.0** |
| Previous ART cycle | | | | | | | | | |
| No | 139 | 2.3 | 1.5–3.8 | 28 | 10.0–65.0 | 70 | 25.4–147.7 | 39 | 26.0–48.0 |
| Yes | 184 | 2.7 | 1.8–3.7 | 33 | 9.7–68.0 | 84 | 29.0–167.5 | 41 | 33.0–50.0 |
| BMI | | | | | | | | | |
| <25.0 | 148 | 2.8 | 1.8–3.9 | 33 | 10.4–63.0 | 84.7 | 28.1–150.0 | 40 | 31.0–48.0 |
| 25.0–29.9 | 150 | 2.4 | 1.5–3.3 | 30 | 9.6–67.5 | 70 | 24.0–151.9 | 42 | 28.0–50.0 |
| ≥30.0 | 28 | 2.7 | 2.0–4.3 | 33 | 8.0–66.0 | 82.5 | 28.1–175.5 | 41 | 31.0–51.0 |
| Smoking | | | | | | | | | |
| Never | 129 | 2.6 | 1.8–4.0 | 30 | 9.7–58.0 | 75.7 | 21.6–140.0 | 38 | 29.0–46.0 |
| Former | 93 | 2.7 | 1.7–3.7 | 30.5 | 10.0–65.5 | 69.8 | 25.5–165.0 | 44 | 34.0–51.0 |
| Current | 101 | 2.6 | 1.7–3.5 | 34.5 | 9.9–70.0 | 83 | 34.5–153.0 | 43 | 32.0–49.0 |
| 0–9 cig/day | 46 | 2.7 | 1.7–3.5 | 35 | 11.0–75.0 | 87.3 | 37.5–142.5 | 41 | 27.0–50.0 |
| ≥10 cig/day | 15 | 2.5 | 1.7–3.3 | 30 | 8.7–69.0 | 80.1 | 21.4–168.0 | 44 | 36.0–49.0 |
| Daily alcohol intake (units/week) | | | | | | | | | |
| Abstainer | 31 | **1.8** | **1.2–2.5** | **42** | **18.0–75.0** | **85.4** | **37.8–151.9** | 41.5 | 32.0–47.5 |
| <1–3 | 97 | **2.4** | **1.7–3.5** | **24.5** | **5.9–50.0** | **51.5** | **15.2–114.7** | 38 | 29.5–46.0 |
| 4–7 | 98 | **3** | **2.0–4.0** | **31** | **8.7–71.0** | **87.9** | **20.2–182.1** | 42 | 32.0–50.0 |
| ≥8 | 97 | **2.6** | **1.5–4.0** | **39** | **16.0–72.0** | **84** | **37.4–156.4** | 42 | 28.0–50.0 |
| Caffeine intake (mg/day) | | | | | | | | | |
| 0–127 | 110 | 2.8 | 1.8–4.0 | 32.5 | 10.0–61.5 | 86.5 | 23.4–151.9 | 39 | 30.5–49.5 |
| 128–214 | 108 | 2.5 | 1.7–3.7 | 31 | 8.7–70.0 | 70.2 | 24.0–156.4 | 41 | 31.0–48.0 |
| ≥215 | 109 | 2.5 | 1.5–3.3 | 30 | 10.1–63.5 | 79.6 | 32.7–149.0 | 42 | 28.0–49.5 |
| Occupational physical activity | | | | | | | | | |
| Heavy | 67 | 2.7 | 1.4–3.7 | 29 | 6.6–65.0 | 54 | 17.6–150.0 | 41 | 36.0–50.0 |
| Light/moderate | 66 | 2.6 | 1.8–4.0 | 30.5 | 12.6–63.5 | 76 | 35.7–156.0 | 43.5 | 32.0–48.5 |
| Mainly standing | 48 | 2.3 | 1.7–3.9 | 40 | 15.9–82.0 | 100.1 | 37.5–180.0 | 41 | 23.0–46.0 |
| Mainly sitting | 145 | 2.7 | 1.8–3.6 | 31.5 | 9.7–64.0 | 79.9 | 21.6–148.0 | 40 | 28.5–50.0 |
| Leisure physical activity | | | | | | | | | |
| <2 h/week | 137 | **2.5** | **1.6–3.1** | 33 | 8.7–66.0 | 70.2 | 17.5–156.4 | **43** | **33.0–51.0** |
| 2–4 h/week | 112 | **2.6** | **1.7–3.9** | 31.5 | 9.9–70.0 | 75.4 | 31.8–156.2 | **37.5** | **25.5–45.5** |
| ≥5 h/week | 74 | **3** | **2.0–4.0** | 32.5 | 11.3–57.0 | 85.9 | 36.1–147.4 | **42** | **30.0–49.0** |

Bold: $p < 0.05$; Kruskal–Wallis test.

units/week), sperm parameters of men with 0 and 1–5 units per week were largely similar, if not better in the latter. In our sample, a relatively low alcohol intake was frequent: although 90% of men reported some alcohol consumption, about one third drank no more than 3 units per week and one third no more than 7 units/week. Therefore, the majority had levels of alcohol intake similar to the lowest consumption category of Jensen *et al.*'s study (Jensen *et al.*, 2014).

A relation between alcohol drinking and semen parameter is biologically plausible. It is known that beer and wine contain polyphenols such as resveratrol or xanthohuminol, which were demonstrated to have a strong therapeutic and cell protective potential (Wogatzky *et al.*, 2012; Cui *et al.*, 2016). Accordingly, it can be suggested that these compounds might stand behind the observed beneficial effects found in this study. On the other hand, different studies experimentally proved that alcohol has a detrimental effect at all levels of the male reproductive system: it interferes with the function of the hypothalamic-pituitary-testicular axis, impairing gonadotropin secretion with consequent decreasing of testosterone levels (Muthusami & Chinnaswamy, 2005; Maneesh *et al.*, 2006). Likewise, the ratio between free estradiol and free testosterone is modified by alcohol consumption (Hansen *et al.*, 2012). Studies on heavy alcohol intake (Kucheria *et al.*, 1985; Muthusami & Chinnaswamy, 2005)

**Table 3** Adjusted median sperm parameters (95% confidence intervals) according to alcohol intake, in strata of risk factor for impaired male fertility and leisure physical activity

| Alcohol intake (units/week) | Overall[a] *N* = 323 | | Risk factor for impaired male fertility[b] | | | | Leisure physical activity (hours/week)[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No *N* = 257 | | Yes *N* = 66 | | <2 *N* = 133 | | 2–4 *N* = 112 | | ≥5 *N* = 74 | |
| Volume (mL) | | | | | | | | | | | | |
| Abstainers | **2.1** | **1.6–2.6** | 1.9 | 1.5–2.4 | 1.8 | 0.7–3.5 | **1.7** | **1.2–2.3** | 2.1 | 1.2–3.3 | 3.1 | 1.8–4.7 |
| <1–3 | 2.7 | 2.4–3.0 | 2.4 | 2.1–2.8 | 3.3 | 2.6–4.1 | 2.6 | 2.2–3.1 | 2.4 | 1.9–3.0 | 3.1 | 2.4–3.9 |
| 4–7 | **3.1** | **2.8–3.5** | 2.8 | 2.5–3.2 | 3.7 | 3.0–4.5 | 2.5 | 2.0–3.1 | **3.2** | **2.6–3.8** | 3.7 | 3.0–4.4 |
| ≥8 | 2.7 | 2.4–3.1 | 2.7 | 2.4–3.0 | 2.4 | 1.8–3.0 | 2.5 | 2.1–3.0 | 2.3 | 1.8–2.9 | 3.7 | 2.9–4.5 |
| Concentration (mil/mL) | | | | | | | | | | | | |
| Abstainers | **36.4** | **22.8–53.1** | 45 | 28.8–64.8 | 66.4 | 22.8–132.7 | 26.9 | 12.2–47.2 | **59.8** | **24.4–110.7** | 29.5 | 7.6–65.7 |
| <1–3 | 19.3 | 13.2–26.4 | 27.5 | 19.6–36.7 | 14.1 | 5.6–26.4 | 19.5 | 10.4–31.5 | 20.9 | 10.4–35.0 | 18.6 | 8.2–33.0 |
| 4–7 | **28.6** | **21.4–36.8** | **42.7** | **33.2–53.4** | 12.3 | 4.5–23.9 | 23.9 | 12.8–38.6 | 33.8 | 21.0–49.6 | 32.2 | 20.1–47.0 |
| ≥8 | **34** | **26.0–43.1** | **47.6** | **37.1–59.3** | 22.4 | 12.1–35.8 | **36.2** | **23.0–52.4** | **40.1** | **25.4–58.1** | 28.7 | 15.5–46.0 |
| Total sperm count (million) | | | | | | | | | | | | |
| Abstainers | 74.6 | 42.6–115.4 | 93.9 | 55.5–142.2 | 127.7 | 33.4–283.1 | 46.3 | 16.8–90.6 | 128.5 | 42.2–261.7 | 78.3 | 20.6–173.2 |
| <1–3 | 48.5 | 32.3–68.0 | 64.9 | 44.7–88.9 | 48.8 | 21.9–86.4 | 41.2 | 20.3–69.3 | 52.1 | 23.0–92.9 | 56.2 | 26.8–96.2 |
| 4–7 | **85.1** | **64.4–108.7** | **117.7** | **91.3–147.3** | 55.5 | 26.9–94.2 | 53.8 | 27.2–89.4 | **105** | **65.5–153.7** | **105.1** | **69.6–147.8** |
| ≥8 | **84.1** | **63.0–108.2** | **121.6** | **93.8–153.0** | 43 | 20.3–74.0 | **80.9** | **49.5–120.0** | 86.8 | 49.9–133.9 | 92.3 | 53.5–141.5 |
| Motility (%) | | | | | | | | | | | | |
| Abstainers | 33.4 | 27.2–40.2 | 37.9 | 31.8–44.7 | 37.3 | 12.8–74.6 | 35.9 | 27.8–45.0 | 30.3 | 17.4–46.7 | 41.2 | 26.3–59.3 |
| <1–3 | 33.4 | 29.6–37.5 | 38.7 | 34.9–42.8 | 28.9 | 18.1–42.3 | 36.8 | 30.8–43.2 | 33.6 | 26.1–42.0 | 32.5 | 25.2–40.8 |
| 4–7 | 35.3 | 31.6–39.3 | 40 | 36.4–43.8 | 33 | 21.6–46.8 | 40.9 | 34.1–48.4 | 29.7 | 23.4–36.6 | 35.1 | 28.6–42.3 |
| ≥8 | 34.9 | 31.1–38.9 | 41.3 | 37.4–45.4 | 28.1 | 19.0–39.0 | 41.4 | 35.1–48.2 | 28.8 | 22.4–35.9 | 34.5 | 26.9–43.1 |

Adjusted medians were calculated back-transforming adjusted means of square-rooted variables and their corresponding 95% CI. Bold: $p < 0.05$ as compared to <1–3 units/week of alcohol intake. [a]Adjusted for age (<35, 35–39, ≥40 years), risk factor for impaired male fertility (no/yes), caffeine (tertiles of daily intake), smoking (never, former, current), leisure physical activity (<2, 2–4, ≥5 h/week), days of abstinence and daily calories intake(as continuous variables). [b]Adjusted for age (<35, 35–39, ≥40 years), caffeine (tertiles of daily intake), smoking (never, former, current), leisure physical activity (<2, 2–4, ≥5 h/week), days of abstinence and daily calories intake(as continuous variables). [c]Adjusted for age (<35, 35–39, ≥40 years), risk factor for impaired male fertility (no/yes), caffeine (tertiles of daily intake), smoking (never, former, current), days of abstinence and daily calories intake (as continuous variables).

related the low semen volume to the testosterone reduction due to alcohol abuse, causing damage to Leydig cells or impairment of hypothalamic-pituitary-gonadal axis. Conversely, Jensen *et al.* (2014) found increasing testosterone levels (total and free) with increasing alcohol intake in young Danish men. With few exceptions, patients in our cohort had a moderate alcohol intake and the detrimental effects, at these levels of consumption, might be balanced by increasing testosterone levels and cell protective potential of resveratrol or xanthohuminol. However, the mechanisms underlying the positive association between moderate alcohol intake and semen parameters, if true, are not easily comprehensible and need to be further investigated.

Some limitations and strengths of our study deserve to be commented.

A first important limitation is that our findings should be referred only to patients of infertile couples.

The information regarding alcohol use was self-reported, thus some misclassification may have occurred. However, studies investigating reproducibility and validity of self-reported alcohol drinking (Flagg *et al.*, 2000; Horn-Ross *et al.*, 2008), in different populations, found satisfactory correlation coefficients (at least 0.61). Furthermore, in Italy alcohol consumption is socially accepted and recommendations to avoid alcohol for fertility preservation are not routinely advocated during assisted reproduction procedures. On the contrary, underreporting of cigarette consumption was possible, due to a lower social acceptability of smoking (Gallus *et al.*, 2011). However, an underreporting should tend to reduce the estimated association between alcohol and semen parameters.

Among the strengths of this study, we mention the relatively large sample size, which is even more significant as this is a single institution study. Men were interviewed in the same Institution by the same personnel, and participation was practically complete. Moreover, we analysed the role of alcohol in men with or without other conditions associated with infertility. We also accounted for potential biases, such as age, smoking, BMI, calories intake, days of abstinence, that have been reported to be associated with semen quality (Li *et al.*, 2011).

In conclusion, in this cohort of male partners of subfertile couples undergoing ART cycles, alcohol intake was not negatively associated with semen quality. In particular, higher semen volume was observed in men with 4–7 units/week of alcohol intake, and ≥8 units/week were not negatively associated with other seminal variables. Patients drinking 4–7 units per week also showed a higher total sperm count in athe subgroup of men with no risk factors for impaired fertility, and in those with 2–4 and ≥5 h/week of leisure physical activity.

Considering the high proportion of moderate drinkers included in our population, we could not analyse the role of heavy or binge drinking, which are consistently associated to detrimental effects on semen quality. Considering that reassuring results of our study were related to moderate intake, all men undergoing assisted reproduction should be advised to limit alcohol consumption. As this study has not addressed all concerns regarding the effect of male drinking on reproduction and fertility, other domains of reproductive outcomes need further investigation.

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## POTENTIAL CONFLICT OF INTEREST

The authors have no competing financial interest to declare.

## AUTHORS' CONTRIBUTIONS

FP and ILV designed the research study; SF, ES and SN contributed to data acquisition and interpretation; ER, SC and VDC analysed the data; ER, SN, ES and FP interpreted the information and wrote the paper.

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