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Pre-treatment maternal lifestyle and outcomes of assisted reproduction: an Italian cohort study.

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Abstract

Objective. We investigated whether lifestyle affects assisted reproduction technique (ART) outcomes.

Design. Prospective cohort study.

Setting. Italian Fertility Unit.

Participants. Women from couples presenting for evaluation and eligible for ART were invited to participate. Information on alcohol intake, current smoking and leisure physical activity during the year before the interview was collected, using a structured questionnaire. We considered the ART outcomes of the cycle immediately following the interview.

Primary and secondary outcome measures. The primary outcome measure was clinical pregnancy rate. Secondary measures were number of retrieved oocytes, implantation and live birth.

Results. Out of 492 cycles, 427 (86.8%) resulted in embryo transfer, 157 (31.9%) in clinical pregnancy, 121 (24.6%) in live birth. In women in the 3rd tertile of alcohol intake, adjusted relative risk (ARR) was 1.32 (95% confidence interval (CI) 0.71-2.47), 1.05 (95% CI 0.89-1.23) and 1.05 (95% CI 0.92-1.19) for implantation, clinical pregnancy and live birth failure respectively. The corresponding figures in women currently smoking more than 5 cigarettes/day were 1.13 (95% CI 0.46-2.80), 1.04 (95% CI 0.84-1.29), and 0.98 (95% CI 0.83-1.16), and in women with physical activity ≥5 hours/week were 1.75 (95% CI 0.91-3.40), 1.11 (95% CI 0.96-1.28), and 1.10 (95% CI 0.95-1.28) respectively.

Conclusion. Conservatively, all women seeking pregnancy should be advised to limit alcohol drinking and, of course, smoking, while maintaining moderate level of physical activity.

Keywords. Alcohol intake, smoking habits, leisure physical activity, assisted reproduction techniques, pregnancy, live birth.

Strengths and limitations of this study

- This study analysed several lifestyle factors of women interviewed in the same institution, participation was practically complete, and information on nutritional status was also available.
- Smoking and drinking habits were self-reported by women, so some underestimates could have occurred.
- Smoking and drinking aside, this study offer information about physical activity in the period preceding assisted reproduction, a factor still under discussion.

Introduction

Alcohol consumption and smoking are among the most common lifestyle exposures in women. During the last decades, the relationship between these lifestyle factors and spontaneous fertility has been investigated in several observational studies: some have shown that alcohol and smoking affect spontaneous fertility (Hassan & Killick, 2004; Oboni, Marques-Vidal, Bastardot, Vollenweider, & Waeber, 2016), although not consistently (Hawkins Bressler et al., 2016; Mikkelsen et al., 2016).

These exposures may contribute to spontaneous reproductive failures, but they may also impair the success rate of assisted reproduction technology (ART). Thus, it is conceivable that modifying such lifestyle habits before treatments could reduce the need for ART procedures and/or enhance the likelihood of in vitro fertilization (IVF) success. In a study conducted in California, alcohol intake was negatively associated with the number of oocytes retrieved, but not with live birth rate (Klonoff-Cohen, Lam-Kruglick, & Gonzalez, 2003). Otherwise, in a prospective cohort study, no association emerged between alcohol intake during days 4–10 of ovarian stimulation and IVF outcomes (Firns et al., 2015). A recent systematic revision of literature confirmed that average alcohol intake before ART initiation did not impact on the outcomes, whereas intake at the start of ART cycle had a negative effect on fertilization, embryo quality and implantation (Mínguez-Alarcón, Chavarro, & Gaskins, 2018).

Cigarette smoking is the most common lifestyle factor that could affect IVF outcomes and several studies have demonstrated the negative effect of smoking on pregnancy rate and upon clinical outcome of ART (Waylen, Metwally, Jones, Wilkinson, & Ledger, 2009). However, literature on this issue is still limited (Hornstein, 2016), although a recent meta-analysis (Budani, Fensore, Di Marzio M, & Tiboni, 2018) found that current smokers undergoing ART had lower clinical pregnancy and live birth rate than non-smokers and experienced a significant increase of spontaneous miscarriage.

Evidence on physical activity (PA) was inconsistent: although data from the Nurses' Health Study II suggested that vigorous activity may reduce ovulatory infertility (Rich-Edwards et al., 2002), a Norwegian cohort indicated that high intensity and frequency of PA increase sub-fertility (Gudmundsdottir, Flanders, & Augestad, 2009). Studies specifically on PA and ART success were equally inconsistent: investigating pre-treatment PA, Morris et al. (Morris et al., 2006) found that women undergoing ART had a 40% reduced likelihood of live birth, if engaged in PA 4 hours or more per week for less than 10 years, compared with women not regularly engaged in PA. Another study (Kucuk, Doymaz, & Urman, 2010) did not find a beneficial effect of activity levels before treatment on clinical outcomes, although moderate PA during ART cycle was associated with higher implantation and live birth rates. Recently, it was suggested that health-promoting lifestyle education may increase the success rates of ART, correcting risk factors that negatively affect fertility (Kaya, Kizilkaya Beji, Aydin, & Hassa, 2016). This evidence has been recently reviewed: pooled estimates from a systematic research found that physical activity before ART cycles was associated with increased rates of clinical pregnancy and live births, but no effect was shown on miscarriage rate (Rao, Zeng, & Tang, 2018).

Alcohol consumption (World Health Organisation (WHO), 2014), smoking habits (Marcon et al., 2018) and physical activity (Gomes et al., 2017; Loyen et al., 2017) largely differ in different populations. Thus, it is interesting to analyse the role of these lifestyles on fertility treatment in an Italian setting, using data from a prospective cohort study conducted in an Italian Fertility Centre.

Methods

From September 2014 to December 2016, in randomly selected days, sub-fertile couples, presenting for evaluation to the Fertility Unit of Fondazione IRCCS Ca' Granda, Ospedale

Maggiore, Policlinico, Milan, and eligible for ART, were invited to participate into a prospective cohort study on the role of lifestyle habits and diet on ART outcomes. The study protocol was approved by the Institutional Ethical 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. The time interval between the proposal of the study and the interview was generally less than one month.

The overall participation rate was close to 95%, mainly since couples were interviewed during the period spent waiting for the different diagnostic stages, before actual ART procedures, and the not sensitive character of questions.

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, anthropometric variables, personal medical history and reproductive history, and lifestyle factors. Couples that cannot speak Italian were excluded from the study.

Patient and public involvement.

Patients were not involved in the design, recruitment or conduct of the study.

The present study reported on the outcome of the cycle immediately following the interview.

Procedures

Patients were asked to report about their usual weekly food consumption in the last year, using a reproducible and valid food frequency questionnaire (Decarli et al., 1996; Franceschi et al., 1995, 1993), including the weekly numbers of drinks for several alcoholic beverages. The questionnaire was satisfactorily reproducible (D'Avanzo, La Vecchia, Katsouyanni,

Negri, & Trichopoulos, 1996). Taking into account the different ethanol concentration, one unit corresponded to approximately 125 ml of wine, 330 ml of beer and 30 ml of hard liquor (i.e., about 12.5 g of ethanol). Total alcohol intake, expressed in grams of ethanol per day (g/day), was computed as the sum of all reported alcoholic beverages. "Never drinkers" and "Ex- drinkers" were patients who abstained from drinking lifelong and for at least 12 months at the time of interview, respectively. For the purpose of this study, we considered these two groups of women in the same category "Abstainers".

A woman was considered a smoker if she had smoked ≥ one cigarette/day for at least one year; a former smoker if she had smoked ≥ one cigarette/day for at least one year, but had stopped more than one year before the interview, and a non-smoker if she had never smoked ≥ one cigarette/day.

The adherence to the Mediterranean diet was assessed through an a priori score (Mediterranean diet score, MDS), developed by Trichopoulou and colleagues (Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003) and calculated as previously published (E. Ricci et al., 2019). To include MDS score in the analysis of alcohol intake, it was recalculated excluding alcohol. Satisfactory reproducibility of questions on self-reported smoking and drinking habits in our study populations has been previously reported (Ferraroni et al., 1996).

Leisure PA was defined as the number of hours per week of a sport or activity such as walking, gardening, cycling, etc., in the year preceding the interview. Scores ranged between 1 and 4, corresponding to <2, 2–4, 5-7 and >7 h of PA per week. No information was available on intensity of activity.

Patients were managed according to a standardized clinical protocol as reported in details elsewhere (Benaglia et al., 2013; Elena Ricci et al., 2018). The choice between conventional IVF or Intra-Cytoplasmatic Sperm Injection (ICSI) was made based on semen characteristics.

Good quality oocytes were those in metaphase I-II for IVF and metaphase II for ICSI. In this analysis, we considered as outcome the best one obtained using the oocytes retrieved in the cycle immediately following the interview. For example, if a woman did not achieve a pregnancy with a fresh embryo transfer, but subsequently a frozen embryo from the same cycle led to a clinical pregnancy, we considered the clinical pregnancy as the main outcome.

All clinical information (including infertility diagnosis) was collected from medical records.

Statistical analysis

Clinical pregnancy was considered the main objective of the study. Considering a 30% of pregnancy rate per cycle, as usual in our Fertility Centre, this study was powered to detect a 1.5 increase of risk in the highest tertile of intake as compared to the lowest (α=0.05, β=0.80).

Multiple outcomes were considered: 1. Number of retrieved good quality oocytes; 2. Embryo transfer; 3. Clinical pregnancy; 4: Live birth. Patients who failed each treatment stage were included in the following stage as failures.

Categorical variables were described as frequency (N) and percentage (%) and compared using the Pearson or Mantel-Haenzsel chi-square, as appropriate. Continuous variables were described as mean and standard deviation (SD) if normally distributed, or median and interquartile range (IQR) if not normally distributed and analysed using analysis of variance and Kruskal-Wallis test respectively.

We used multivariable generalized linear mixed models to evaluate the association of exposure variables with treatment outcomes. We used a Poisson distribution and log link function for the number of good quality oocytes retrieved, and binomial distribution and logit link function for clinical outcomes. We estimated relative risks (RRs) of each clinical outcome and corresponding 95% CIs in categories of alcohol intake (approximate tertiles), former and current smoking (no, ≤ 5, >5 cigarettes/day) and leisure PA (<2, 2-4, ≥5 hours/week) in the year before the interview.

To account for potential confounders, we included terms for variables, that were associated with these modifiable lifestyles, and/or with at least one ART outcome, in the general linear model and multiple log-binomial regression models (as indicated in table footnotes). Terms for interaction were tested.

All the analyses were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

From September 2014 to December 2016, out of 501 women undergoing ART cycle, 9 (1.8%) did not provide complete information about their lifestyle, or were lost to follow-up, and were excluded from this analysis. Analysis was then performed on 492 ART cycle outcomes from 492 women.

Mean age was 36.6 years (standard deviation, SD, 3.6 range 27-45) and mean body mass index (BMI) was 22.3 kg/m^2 (SD 3.9, range 16.4-41.7). Thirty women (6.1%) were obese (BMI $\geq$ 30.0 kg/m^2).

The characteristics of women according to alcohol, smoking habits and PA are shown in Table 1.

Only 16 women (3.2%) exercised more than 7 hours per week, so we merged the two categories 5-7 and >7 hours/week. Leisure PA was associated with college degree and higher MDS, and inversely with daily calories intake.

Of the 492 initiated cycles, 427 (86.8%) resulted in embryo transfer, 157 (31.9%) in clinical pregnancy, 121 (24.6%) in live births. Out of 36 clinical pregnancies not resulting in live birth, 34 ended with miscarriage, one with an induced abortion, and one was extra-uterine.

Age was the main risk factor for ART failure. The median of good quality oocytes was 6 (IQR 4-9) in women <35 years old, 5 (IQR 3-8) in 35-39, 3 (IQR 2-6) in women aged $\geq$ 40

years (p<0.0001). No association was observed at univariate analysis with alcohol intake, current smoking or leisure PA. As compared to women aged < 35, RR for not achieving embryo transfer was 2.01 (95% CI 1.03-3.93) for women aged 35-39 and 2.29 (95% CI 1.11-4.72) for those aged ≥40 years. The corresponding figures were 1.16 (95% CI 0.98-1.37) and 1.40 (95% CI 1.18-1.66) for clinical pregnancy; 1.19 (95% CI 1.03-1.37) and 1.37 (95% CI 1.19-1.78) for live birth.

At univariate analysis, leisure PA ≥ 5 hours/week was significantly associated with higher risk of not achieving clinical pregnancy (RR 1.25, 95% CI 1.08-1.45), whereas no relationship was observed with embryo transfer and live birth. Alcohol intake and current smoking were not significantly associated with any ART outcomes.

Table 2 shows the relation between exposures and clinical results, accounting for potential confounders. No significant association was observed between smoking, alcohol intake and leisure PA, thus they were not mutually adjusted. Terms for interaction between smoking, alcohol intake and leisure PA did not show any significance and were excluded from the final models.

In this sample, 28 (5.7%) women drank at least 1 alcohol unit per day. Although the ARR for embryo transfer failure was higher, it was not significant as compared to abstainers, and no effect was observed on other outcomes. ARR for embryo transfer failure was significantly higher in six women who smoked ≥20 cigarettes/day during the year before ART procedure. Former smokers who stopped smoking more than 5 years before undergoing ART had a higher number of oocytes than never smokers.

Finally, we controlled these results for partner's lifestyle, in a subgroup of 324 couples with complete information for both male and female. As regards to women lifestyle, results did not change. Men's lifestyle (smoking, alcohol drinking and physical activity) did not significantly impact on ART outcomes.

Discussion

In this sample of women referring to an Italian Fertility centre, lifestyle habits did not play a significant role in the outcome of ART, except for heavy smoking, that was associated with fewer good quality oocytes and worse outcome of embryo transfer.

Alcohol

The role of alcohol intake on spontaneous fertility has been associated with decreased fertility and chance of conception (Eggert, Theobald, & Engfeldt, 2004; Jensen et al., 1998; Mikkelsen et al., 2016). A recent meta-analysis of observational studies, including about 100,000 women, suggested that alcohol consumption was associated with reduced fecundability (Fan et al., 2017). In biological terms, alcohol may lower fertility affecting endogenous hormone levels (Rossi et al., 2011) and embryo quality (Wdowiak, Sulima, Sadowska, Bakalczuk, & Bojar, 2014).

The role of alcohol intake on ART success rate has been analysed in some studies showing inconsistent results (Abadia et al., 2017; Firns et al., 2015; Gormack et al., 2015; Klonoff-Cohen et al., 2003). These differences may be partially due to the high heterogeneity between studies, in particular in terms of intake prevalence. For example, in Boston, US, a study, found that 30% of women reporting >1 unit/day of alcohol intake had an increased risk of adverse outcome, but no association between low to moderate alcohol consumption was observed (Abadia et al., 2017). Literature suggests that alcohol drinking at start of ART may exert a detrimental effect, but the evidence is still limited (Mínguez-Alarcón et al., 2018).

Along this line, we did not find any association between alcohol intake and ART outcomes, but we were not able to analyse the effect of high alcohol intake, present in only 5.7% of our sample.

Smoking

Since it appears to have a detrimental effect on spontaneous fertility (Dechanet et al., 2011; Hassan & Killick, 2004), it has been suggested that cigarette smoking may affect IVF outcomes as well.

However, individual studies available on this issue do not always support a significant association between smoking and IVF success or oocyte quality. For example, in a study conducted in Australia in women undergoing IVF (Firns et al., 2015), the mean number of oocytes did not significantly differ between regular smokers (11.1, SD 6.5), ex-smokers (11.8, SD 10.1) or non-smokers (11.2, SD 7.6), indicating that smoking might not influence oocytes production. Interestingly, the same analysis showed that fertilization rates were not influenced by current smoking status but decreased as years of smoking increased ($p < 0.001$).

In a large Dutch nationwide retrospective analysis conducted on 8457 patients (Lintsen et al., 2005), no significant difference was observed in the mean number of oocytes retrieved between non-smokers and smokers, yet there were significantly lower clinical pregnancy and live birth rates for smoking patients.

These findings regarding clinical pregnancies and live birth rate were further confirmed by more recent meta-analysis and reviews (Hornstein, 2016; Waylen et al., 2009). As regards to former smokers, they tend to have better ART outcomes than current smokers (Mínguez-Alarcón et al., 2018), but the evidence is scanty about the influence of smoking cessation on ART outcomes.

Vanegas et al. suggested that male smoking could also be associated with ART outcomes (Vanegas et al., 2017). Whereas male current smoking did not affect the different stages of ART (egg retrieval, fertilization, embryo transfer, implantation, clinical pregnancy and livebirth), among past smokers every additional year since a man had quit smoking reduced the risk of failing ART by 4%, particularly between clinical pregnancy and live birth.

In our sample, good quality oocytes number was significantly lower in women who had smoked 20 or more cigarettes per day in the year before ART procedure, as compared to never smokers as well as to women currently smoking 5 or less cigarettes/day, and risk of embryo transfer failure was significantly higher than in never smokers. No significant effect was observed on clinical pregnancy and live birth. However, only six women had such a high level of smoking, thus our estimates should be considered with caution. Male smoking did not appear as a contributing factor to ART failure.

Leisure Physical Activity

Findings from literature are inconsistent. Among 2,232 patients prospectively enrolled before their first IVF cycle, Morris et al. (Morris et al., 2006) found that women who exercised 4 or more hours per week, for 1-9 years before ART cycle, were more likely to experience an implantation failure (OR 2.0, 95% CI 1.4-3.1) or pregnancy loss (OR 2.0, CI 1.2-3.4) than women who did not report exercise.

On the contrary, in a group of 131 women (Kucuk et al., 2010) those who were physically more active during the ART procedure were more likely to have an increased implantation rate and a live birth; none of these women met the criteria for high PA, so that the comparison was done between low and moderate PA. On the same line, an observational study (Evenson et al., 2014) found that a self-reported active lifestyle in the preceding year affected favourably the ART outcome in 121 women, with clinical pregnancy more likely in women with a level above median for each kind of activity: active living (OR 1.96, 95% CI 1.09-3.50), sports/exercise (OR 1.48, 95% CI 1.02–2.15), and total activity (OR 1.52, 95% CI 1.15–2.01). Recently, findings from the EARTH Study (Gaskins et al. 2016) suggested that time spent in moderate-to-vigorous physical activities before IVF was not associated with probability of implantation, clinical pregnancy or live birth, in 273 women who underwent

427 IVF cycles. Pooled estimates from a recent meta-analysis (Rao et al., 2018) suggested a beneficial effect of PA on ART outcomes, and no effect on spontaneous abortion; despite this, study results are widely heterogeneous.

In our sample, an increased risk of embryo transfer failure was suggested in women with ≥5 hours/week of leisure PA, but this result was not significant in the multivariable analysis.

Strengths and Limitations.

Potential limitations should be considered. All information on smoking and drinking was self-reported by women, so some underestimates could have occurred. However, in Italy, alcohol consumption is socially accepted and recommendations to avoid alcohol to protect fertility have not received widespread attention and are not routinely advocated by gynaecologists before IVF.

Other sources of bias, including selection or confounding factors, are also unlikely to have produced marked effects, especially considering that all women were interviewed in the same institution and that participation was practically complete. Moreover, we analysed information on nutritional status, and their inclusion into the model did not change the estimated RRs.

Comparing the clinical pregnancy percentage in women who did not drink at all and those in the 3rd tertile of intake, the power of detecting a significant difference was about 13%. Using our data, with 30% prevalence of abstainers, we could identify a RR of not achieving clinical pregnancy of 1.8 for drinkers.

Lastly, this study only included women presenting for ART, thus the findings are not generalizable to the wider population.

Conclusions

Our study did not show an effect of alcohol consumption, current smoking and leisure physical activity on number of good quality oocytes and success rate after ART procedures.

Considering that reassuring results of our study were related to moderate alcohol intake and cigarette smoking, conservatively, all women seeking pregnancy should be advised to limit or avoid alcohol drinking and, of course, smoking. Moderate physical activity as a part of a healthy lifestyle is also advisable, although current knowledge does not support convincing evidence of a direct beneficial effect.

Declarations

Ethics approval and consent to participate.

The study protocol was approved by the Ethical Review Board of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano Area B (reference number 2616, 1421/2014).

All patients included gave their written informed consent to participate in the study.

Availability of data and materials.

The dataset analysed during the current study is available from the corresponding author on reasonable request.

Competing interests.

The authors declare that they do not have any competing interests.

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No funding was obtained for this study.

Authors' contribution

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

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Table 1. Demographic characteristics of 492 women, according to alcohol intake, smoking habits and leisure physical activity.

	Alcohol intake (g/day)								Smoking						Leisure Physical Activity					
	Abstainers 0		1st tertile 0.01-2.27		2nd tertile 2.28-5.74		3rd tertile ≥5.75		Never		Current		Former		< 2 hours/week		2-4 hours/week		≥5 hours/week	
	N=140	28.5%	N=117	23.8%	N=122	24.8%	N=113	23.0%	N=272	55.3%	N=90	18.3%	N=130	26.4%	N=256	54.1%	N=175	35.6%	N=51	10.4%
Age (years)																				
<35	**46**	**32.9**	**38**	**32.5**	**25**	**20.5**	**27**	**23.9**	71	26.1	28	31.1	38	29.2	78	29.3	47	26.9	11	21.6
35-39	**70**	**50.0**	**50**	**42.7**	**64**	**52.5**	**59**	**52.2**	131	48.2	46	51.1	65	50.0	123	46.2	89	50.9	31	60.8
≥40	**24**	**17.1**	**29**	**24.8**	**33**	**27.0**	**27**	**23.9**	70	25.7	16	17.8	27	20.8	65	24.4	39	22.3	9	17.6
College degree	**49**	**35.0**	**64**	**54.7**	**70**	**57.4**	**72**	**63.7**	**151**	**55.5**	**35**	**28.5**	**66**	**50.8**	**120**	**45.1**	**110**	**62.9**	**25**	**49.0**
Cause of infertility																				
Male factor only	37	26.4	35	29.9	33	27.0	23	20.4	**69**	**25.4**	**22**	**24.4**	**37**	**28.5**	68	25.6	47	26.9	13	25.5
Low ovarian reserve	26	18.6	25	21.4	24	19.7	22	19.5	**55**	**20.2**	**20**	**22.2**	**22**	**16.9**	51	19.2	36	20.6	10	19.6
Endometriosis	27	19.3	21	18.0	28	23.0	27	23.9	**52**	**19.1**	**19**	**21.1**	**32**	**24.6**	64	24.1	29	16.6	10	19.6
Ovulatory	10	7.1	5	4.3	4	3.3	0	0	**15**	**5.5**	**0**	**0**	**4**	**3.1**	12	4.5	5	2.9	2	3.9
Tubal	11	7.9	17	14.5	11	9.0	15	13.3	**19**	**7.0**	**16**	**17.8**	**19**	**14.6**	26	9.8	21	12.0	7	13.7
Unexplained	29	20.7	14	12.0	22	18.0	26	23.0	**62**	**22.8**	**13**	**14.4**	**16**	**12.3**	45	16.9	37	21.1	9	17.6
BMI (Kg/m^{-2})																				
<18.5	**12**	**8.6**	**11**	**9.4**	**12**	**9.8**	**12**	**10.6**	28	10.3	8	8.9	11	8.5	28	10.5	15	8.6	4	7.8
18.5-24.9	**98**	**70.0**	**80**	**68.4**	**97**	**79.5**	**94**	**83.2**	202	74.3	67	74.4	100	76.9	190	71.4	137	78.3	42	82.4
25.0-29.9	**16**	**11.4**	**15**	**12.8**	**8**	**6.6**	**7**	**6.2**	26	9.6	7	7.8	13	10.0	29	10.9	13	7.4	4	7.8
≥30.0	**14**	**10.0**	**11**	**9.4**	**5**	**4.1**	**0**	**0**	16	5.9	8	8.9	6	4.6	19	7.1	10	5.7	1	2.0
Occupational PA																				

Heavy/moderate	**50**	**35.7**	**27**	**23.3**	**33**	**27.1**	**28**	**24.8**	70	25.7	26	28.9	42	32.3	83	31.3	40	22.9	15	30.0
Mainly standing	**32**	**22.9**	**30**	**25.9**	**25**	**20.5**	**19**	**16.8**	58	21.3	22	24.4	26	20.0	60	22.6	32	18.3	14	28.0
Mainly sitting	**58**	**41.4**	**59**	**50.9**	**63**	**51.6**	**66**	**58.4**	144	52.9	42	46.7	60	46.2	122	46.1	103	58.9	21	42.0
Previous ART cycle	84	60.0	67	57.3	69	56.6	65	57.5	165	60.7	42	46.7	78	60.0	155	58.3	98	56.0	32	62.8
Mean calories (Kcal/day), mean (SD)	1711	(458)	1705	(401)	1782	(415)	1812	(493)	1764	(437)	1761	(446)	1712	(457)	**1788**	**(444)**	**1739**	**(425)**	**1597**	**(481)**
Mediterranean diet Score (n=473)*																				
0-4	51	38.4	37	32.5	33	28.0	34	31.5	75	28.6	32	37.2	25	20.0	**79**	**31.1**	**41**	**24.3**	**12**	**24.5**
5-6	53	39.8	52	45.6	53	44.9	45	41.7	113	43.1	31	36.1	56	44.8	**110**	**43.3**	**73**	**43.2**	**16**	**32.6**
7-9	29	21.8	25	21.9	32	27.1	29	26.8	74	28.2	23	26.7	44	35.2	**65**	**25.6**	**55**	**32.5**	**21**	**42.9**

Bold: p<0.05; sometimes the sums do not add up to the total because of missing values

ART: assisted reproduction technique; PA: physical activity; SD: standard deviation

*MDS without alcohol was calculated for class of alcohol intake

Table 2. Relative risks for failure in clinical outcomes of ART, in 492 women according to alcohol, smoking habits and leisure physical activity.

	N	Number of high-quality oocytes (median, Q1-Q3)	Embryo transfer Failure		ARR (95% CI)	Clinical pregnancy Failure		ARR (95% CI)	Live birth Failure		ARR (95% CI)
Alcohol intake			N	%		N	%		N	%	
Abstainers	140	5 (3-8)	16	11.4	1	92	65.7	1	102	72.9	1
1st tertile	117	4 (2-8)	14	12.0	1.08 (0.55-2.12)	72	61.5	0.95 (0.80-1.12)	84	71.8	1.00 (0.89-1.13)
2nd tertile	122	4 (3-7)	16	13.1	1.02 (0.53-1.96)	91	74.6	1.10 (0.93-1.29)	98	80.3	1.07 (0.95-1.21)
3rd tertile	113	5 (3-8)	19	16.8	1.32 (0.71-2.47)	80	70.8	1.05 (0.89-1.23)	87	77.0	1.05 (0.92-1.19)
≥1 drink/day	28	5 (3-8)	5	17.8	1.34 (0.53-3.40)	19	67.9	1.05 (0.78-1.41)	20	71.4	1.01 (0.76-1.34)
Current smoking											
Never	272	5 (3-8)	34	12.5	1	187	68.8	1	209	76.8	1
Current	91	5 (3-8)	13	14.4	1.42 (0.77-2.60)	64	71.1	1.08 (0.91-1.28)	68	75.6	1.03 (0.88-1.20)
Former	130	5 (3-8)	18	13.8	1.15 (0.68-1.94)	84	64.6	0.98 (0.86-1.11)	94	72.3	0.94 (0.84-1.08)
Current ≤ 5 cig/day	42	5 (3-7)	8	19.0	1.70 (0.84-3.43)	32	74.4	1.11 (0.88-1.41)	34	79.1	1.06 (0.88-1.27)
Current > 5 cig/day	48	4 (3-8)	5	10.4	1.13 (0.46-2.80)	32	68.1	1.04 (0.84-1.29)	34	72.3	0.98 (0.83-1.16)
Current ≥20 cig/day	6	3 (2-6)*	2	33.3	5.20 (1.40-19.2)	5	83.3	1.00 (0.69-1.73)	5	83.3	1.08 (0.65-1.79)

Former (1-5 years)	75	5 (3-9)	14	18.7	1.62 (0.92-2.84)	51	68.0	1.02 (0.87-1.19)	56	74.7	0.99 (0.87-1.12)
Former (>5 years)	55	5 (2-9)**	4	7.3	0.57 (0.21-1.54)	33	60.0	0.92 (0.76-1.12)	38	69.1	0.94 (0.82-1.09)
Leisure PA											
<2 h/wk	266	5 (3-8)	35	13.2	1	179	67.3	1	199	74.8	1
2-4	175	4 (3-8)	20	11.4	0.83 (0.48-1.43)	113	64.6	0.99 (0.90-1.08)	128	73.1	0.99 (0.91-1.09)
≥5	51	4 (2-8)	10	19.6	1.75 (0.91-3.40)	43	84.3	1.11 (0.96-1.28)	44	86.3	1.10 (0.95-1.28)

ARR: adjusted relative risk; CI: confidence interval; PA: physical activity; the final model included age class, college degree, BMI class (<25.0, ≥25.0), occupational PA, previous ART cycles, calories intake. Cause for infertility for smoking habits, and MDS score for PA were also included.

* p=0.047 as compared to never smokers

** p=0.003 as compared to never smokers

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title and abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	p.2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	p. 4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	p.5
Methods			
Study design	4	Present key elements of study design early in the paper	p. 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	p. 5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	p. 6-7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	p. 6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	p. 6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	p. 6-7
Bias	9	Describe any efforts to address potential sources of bias	p.8
Study size	10	Explain how the study size was arrived at	p. 8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	p.8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	p.8
		(*b*) Describe any methods used to examine subgroups and interactions	p.8
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods	-

		taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	No sensitivity analyses

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	p.9
		(b) Give reasons for non-participation at each stage	n.a.
		(c) Consider use of a flow diagram	n.a.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	p.9
		(b) Indicate number of participants with missing data for each variable of interest	p.9
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) *Implicit: for each women, the time between ART initiation and outcome (embryo transfer, clinical pregnancy and livebirth)*	-
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*p.9*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	p.9-10 Table 1 and 2
		(*b*) Report category boundaries when continuous variables were categorized	p.9-10 Table 1 and 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	p. 9
Discussion			
Key results	18	Summarise key results with reference to study objectives	p.11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p.14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	p.11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	p.14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	p. 16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Pre-treatment maternal lifestyle and outcomes of assisted reproduction: an Italian cohort study.

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Abstract

Objective. We investigated whether lifestyle affects assisted reproduction technique (ART) outcomes.

Design. Prospective cohort study.

Setting. Italian Fertility Unit.

Participants. From September 2014 to December 2016, women from couples presenting for evaluation and eligible for ART were invited to participate. Information on alcohol intake, current smoking and leisure physical activity during the year before the interview was collected, using a structured questionnaire. We considered the ART outcomes of the cycle immediately following the interview.

Primary and secondary outcome measures. The primary outcome measure was clinical pregnancy. Secondary measures were number of retrieved oocytes, embryo transfer and live birth.

Results., In 492 women undergoing an ART cycle, 427 (86.8%) underwent embryo transfer, 157 (31.9%) had clinical pregnancy, 121 (24.6%) had live birth. In women in the 3rd tertile of alcohol intake, adjusted relative risk (ARR) was 1.32 (95% confidence interval (CI) 0.71-2.47), 1.05 (95% CI 0.89-1.23) and 1.05 (95% CI 0.92-1.19) for implantation, clinical pregnancy and live birth failure respectively. The corresponding figures in women currently smoking more than 5 cigarettes/day were 1.13 (95% CI 0.46-2.80), 1.04 (95% CI 0.84-1.29), and 0.98 (95% CI 0.83-1.16), and in women with physical activity ≥5 hours/week were 1.75 (95% CI 0.91-3.40), 1.11 (95% CI 0.96-1.28), and 1.10 (95% CI 0.95-1.28) respectively.

Conclusion. Conservatively, all women seeking pregnancy should be advised to limit alcohol drinking and smoking. Moreover, our study suggested that maintaining a moderate level of physical activity could be beneficial.

Keywords. Alcohol intake, smoking habits, leisure physical activity, assisted reproduction techniques, pregnancy, live birth.

Strengths and limitations of this study

- This study analysed several lifestyle factors of women interviewed in the same institution, participation was practically complete, and information on nutritional status was also available.
- Smoking and drinking habits were self-reported by women, so some underestimates could have occurred.
- Smoking and drinking aside, this study offer information about physical activity in the period preceding assisted reproduction, a factor still under discussion.

Introduction

Alcohol consumption and smoking are among the most common lifestyle exposures in women. During the last decades, the relationship between these lifestyle factors and spontaneous fertility has been investigated in several observational studies: some have shown that alcohol and smoking affect spontaneous fertility [1,2], although not consistently [3,4].

These exposures may contribute to spontaneous reproductive failures, but they may also impair the success rate of assisted reproduction technology (ART). Thus, it is conceivable that modifying such lifestyle habits before treatments could reduce the need for ART procedures and/or enhance the likelihood of in vitro fertilization (IVF) success. In a study conducted in California, alcohol intake was negatively associated with the number of oocytes retrieved, but not with live birth rate [5]. Otherwise, in a prospective cohort study, no association emerged between alcohol intake during days 4–10 of ovarian stimulation and IVF outcomes [6]. A recent systematic revision of literature confirmed that average alcohol intake before ART initiation did not impact on the outcomes, whereas intake at the start of ART cycle had a negative effect on fertilization, embryo quality and implantation [7].

Cigarette smoking is the most common lifestyle factor that could affect IVF outcomes and several studies have demonstrated the negative effect of smoking on pregnancy rate and upon clinical outcome of ART [8]. However, literature on this issue is still limited [9], although a recent meta-analysis [10] found that current smokers undergoing ART had lower clinical pregnancy and live birth rate than non-smokers and experienced a significant increase of spontaneous miscarriage.

Evidence on physical activity (PA) was inconsistent: although data from the Nurses' Health Study II suggested that vigorous activity may reduce ovulatory infertility [11], a Norwegian cohort indicated that high intensity and frequency of PA increase sub-fertility [12]. Studies specifically on PA and ART success were equally inconsistent: investigating pre-treatment

PA, Morris et al. [13] found that women undergoing ART had a 40% reduced likelihood of live birth, if engaged in PA 4 hours or more per week for less than 10 years, compared with women not regularly engaged in PA. Another study [14] did not find a beneficial effect of activity levels before treatment on clinical outcomes, although moderate PA during ART cycle was associated with higher implantation and live birth rates. Recently, it was suggested that health-promoting lifestyle education may increase the success rates of ART, correcting risk factors that negatively affect fertility [15]. This evidence has been recently reviewed: pooled estimates from a systematic research found that physical activity before ART cycles was associated with increased rates of clinical pregnancy and live births, but no effect was shown on miscarriage rate [16].

These factors have been analysed in relation to sperm quality. Several reviews showed that the same lifestyle habits, such as smoking and regular drinking, negatively impacting female fertility, also have a detrimental effect on semen quality [17,18]. On the contrary, the effect of physical activity is still under discussion [19,20].

Alcohol consumption [21], smoking habits [22] and physical activity [23,24] largely vary in different populations. Thus, it is interesting to analyse the role of these lifestyles on fertility treatment in an Italian setting, using data from a prospective cohort study conducted in an Italian Fertility Centre.

Methods

From September 2014 to December 2016, in randomly selected days, sub-fertile couples, presenting for evaluation to the Fertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore, Policlinico, Milan, and eligible for ART, were invited to participate into a prospective cohort study on the role of lifestyle habits and diet on ART outcomes. The study

protocol was approved by the Institutional Ethical 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. The time interval between the proposal of the study and the interview was generally less than one month.

The overall participation rate was close to 95%, mainly since couples were interviewed during the period spent waiting for the different diagnostic stages, before actual ART procedures, and the not sensitive character of questions.

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, anthropometric variables, personal medical history and reproductive history, and lifestyle factors. Couples that cannot speak Italian were excluded from the study.

Patient and public involvement.

Patients were not involved in the design, recruitment or conduct of the study.

The present study reported on the outcome of the cycle immediately following the interview.

Procedures

To evaluate the effect of recent exposure, patients were asked to report about their usual weekly food consumption in the last year, using a reproducible and valid food frequency questionnaire [25–27], including the weekly numbers of drinks for several alcoholic beverages. The questionnaire was satisfactorily reproducible [28]. Taking into account the different ethanol concentration, one unit corresponded to approximately 125 ml of wine, 330 ml of beer and 30 ml of hard liquor (i.e., about 12.5 g of ethanol). Total alcohol intake,

expressed in grams of ethanol per day (g/day), was computed as the sum of all reported alcoholic beverages. “Never drinkers” and “Ex- drinkers” were patients who abstained from drinking lifelong and for at least 12 months at the time of interview, respectively. For the purpose of this study, we considered these two groups of women in the same category “Abstainers”.

A woman was considered a smoker if she had smoked ≥ one cigarette/day for at least one year; a former smoker if she had smoked ≥ one cigarette/day for at least one year, but had stopped more than one year before the interview, and a non-smoker if she had never smoked ≥ one cigarette/day.

Before starting ovarian stimulation, women were advised to abstain from alcohol and smoking, thus no such exposure should occur during ART cycle.

The adherence to the Mediterranean diet was assessed through an a priori score (Mediterranean diet score, MDS), developed by Trichopoulou and colleagues [29] and calculated as previously published [30]. To include MDS score in the analysis of alcohol intake, it was recalculated excluding alcohol. Satisfactory reproducibility of questions on self-reported smoking and drinking habits in our study populations has been previously reported [31].

Leisure PA was defined as the number of hours per week of a sport or activity such as walking, gardening, cycling, etc., in the year preceding the interview. Scores ranged between 1 and 4, corresponding to <2, 2–4, 5-7 and >7 h of PA per week. No information was available on intensity of activity.

Patients were managed according to a standardized clinical protocol as reported in details elsewhere [32,33]. The choice between conventional IVF or Intra-Cytoplasmatic Sperm Injection (ICSI) was made based on semen characteristics. Good quality oocytes were those in

metaphase I-II for IVF and metaphase II for ICSI. In this analysis, we considered as outcome the cumulative pregnancy rate per retrieval in the cycle immediately following the interview. For example, if a woman did not achieve a pregnancy with a fresh embryo transfer, but subsequently a frozen embryo from the same cycle led to a clinical pregnancy, we considered the clinical pregnancy as the outcome.

All clinical information (including infertility diagnosis) was collected from medical records.

Statistical analysis

Clinical pregnancy was considered the main objective of the study. Considering a 30% of pregnancy rate per cycle, as usual in our Fertility Centre, this study was powered to detect a 1.5 increase of risk in the highest tertile of intake as compared to the lowest (α=0.05, β=0.80). Multiple outcomes were considered: 1. Number of retrieved good quality oocytes; 2. Embryo transfer; 3. Clinical pregnancy; 4: Live birth. Patients who failed each treatment stage were included in the following stage as failures.

Categorical variables were described as frequency (N) and percentage (%) and compared using the Pearson or Mantel-Haenzsel chi-square, as appropriate. Continuous variables were described as mean and standard deviation (SD) if normally distributed, or median and interquartile range (IQR) if not normally distributed and analysed using analysis of variance and Kruskal-Wallis test respectively. Correlations were evaluated using Pearson r or Spearman rho coefficients, as appropriate.

We used multivariable generalized linear mixed models to evaluate the association of exposure variables with treatment outcomes. We used a Poisson distribution and log link function for the number of good quality oocytes retrieved, and binomial distribution and logit link function for clinical outcomes. We estimated relative risks (RRs) of each clinical outcome and corresponding 95% CIs in categories of alcohol intake (approximate tertiles),

former and current smoking (no, ≤ 5, >5 cigarettes/day) and leisure PA (<2, 2-4, ≥5 hours/week) in the year before the interview.

To account for potential confounders, we included terms for variables, that were associated with these modifiable lifestyles, and/or with at least one ART outcome, in the general linear model and multiple log-binomial regression models (as indicated in table footnotes). Terms for interaction were tested.

All the analyses were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

From September 2014 to December 2016, out of 501 women undergoing ART cycle, 9 (1.8%) did not provide complete information about their lifestyle, or were lost to follow-up, and were excluded from this analysis. Analysis was then performed on 492 ART cycle outcomes from 492 women.

Mean age was 36.6 years (standard deviation, SD, 3.6 range 27-45) and mean body mass index (BMI) was 22.3 kg/m^2 (SD 3.9, range 16.4-41.7). Thirty women (6.1%) were obese (BMI ≥ 30.0 kg/m^2).

The characteristics of women according to alcohol, smoking habits and PA are shown in Table 1.

Only 16 women (3.2%) exercised more than 7 hours per week, so we merged the two categories 5-7 and >7 hours/week. Leisure PA was associated with college degree and higher MDS, and inversely with daily calories intake.

Of the 492 initiated cycles in each woman, 427 (86.8%) resulted in embryo transfer, 157 (31.9%) in clinical pregnancy, 121 (24.6%) in live births. Out of 36 clinical pregnancies not

resulting in live birth, 34 ended with miscarriage, one with an induced abortion, and one was extra-uterine.

Age was the main risk factor for ART failure. The median of good quality oocytes was 6 (IQR 4-9) in women <35 years old, 5 (IQR 3-8) in 35-39, 3 (IQR 2-6) in women aged ≥ 40 years (p<0.0001). No association was observed at univariate analysis with alcohol intake, current smoking or leisure PA. As compared to women aged < 35, RR for missed embryo transfer was 2.01 (95% CI 1.03-3.93) for women aged 35-39 and 2.29 (95% CI 1.11-4.72) for those aged ≥40 years. The corresponding figures were 1.16 (95% CI 0.98-1.37) and 1.40 (95% CI 1.18-1.66) for clinical pregnancy failure; 1.19 (95% CI 1.03-1.37) and 1.37 (95% CI 1.19-1.78) for live birth failure.

At univariate analysis, leisure PA ≥ 5 hours/week was significantly associated with higher risk of not achieving clinical pregnancy (RR 1.25, 95% CI 1.08-1.45), whereas no relationship was observed with embryo transfer and live birth. Alcohol intake and current smoking were not significantly associated with any ART outcomes.

Table 2 shows the relation between exposures and clinical results, accounting for potential confounders. No significant association was observed between smoking, alcohol intake and leisure PA, thus they were not mutually adjusted. Terms for interaction between smoking, alcohol intake and leisure PA did not show any significance and were excluded from the final models.

In this sample, 28 (5.7%) women drank at least 1 alcohol unit per day. Although the ARR for embryo transfer failure was higher, it was not significant as compared to abstainers, and no effect was observed on other outcomes. ARR for embryo transfer failure was significantly higher in six women who smoked ≥20 cigarettes/day during the year before ART procedure. Former smokers who stopped smoking more than 5 years before undergoing ART had a higher number of oocytes than never smokers.

Analysing alcohol intake (g) and number of cigarettes as continuous variables, we did not find any significant correlation with number of high-quality oocytes. Since most women did not smoke, median intakes were 0 (IQR 0-0) in all categories and no differences could be observed. As regards alcohol, women who underwent embryo transfer had median intakes lower than those who did not (1.9 (IQR 0-5.3) vs. 2.7 (IQR 0-7.5 g/day), p=0.16); those who achieved clinical pregnancy consumed 1.8 (IQR 0-4.7) g/day and those who did not achieve it consumed 2.4 (IQR 0-5.6) g/day (p=0.11). Women with livebirth had lower alcohol intake than those without livebirth (1.8 (IQR 0-5.3) vs 2.3 (IQR 0-5.6) g/day, p=0.20). None of these differences was statistically significant.

Considering ICSI and IVF separately, ART outcomes were similar, and including this variable in the equations did not affect the risk estimation.

Finally, we controlled these results for partner's characteristics and lifestyle, in a subgroup of 324 couples with complete information for both male and female. Men's age was significantly associated with higher rate of negative outcomes in the univariate analysis, but when including women's age in the model this relationship lost significance. As regards to women lifestyle, results did not change. Men's lifestyle (smoking, alcohol drinking and physical activity) did not significantly impact on ART outcomes.

Discussion

In this sample of women referring to an Italian Fertility centre, lifestyle habits did not play a significant role in the outcome of ART, except for heavy smoking, that was associated with fewer good quality oocytes and higher rate of missed embryo transfer.

Alcohol

The role of alcohol intake on spontaneous fertility has been associated with decreased fertility and chance of conception [4,34,35]. A recent meta-analysis of observational studies,

including about 100,000 women, suggested that alcohol consumption was associated with reduced fecundability [36]. In biological terms, alcohol may lower fertility affecting endogenous hormone levels [37] and embryo quality [38].

The role of alcohol intake on ART success rate has been analysed in some studies showing inconsistent results [5,6,39,40]. These differences may be partially due to the high heterogeneity between studies, in particular in terms of intake prevalence. For example, in Boston, US, a study, found that 30% of women reporting >1 unit/day of alcohol intake had an increased risk of adverse outcome, but no association between low to moderate alcohol consumption was observed [39]. Literature suggests that alcohol drinking at start of ART may exert a detrimental effect, but the evidence is still limited [7].

Along this line, we did not find any association between alcohol intake and ART outcomes, but we were not able to analyse the effect of high alcohol intake, present in only 5.7% of our sample.

Smoking

Since it appears to have a detrimental effect on spontaneous fertility [1,41], it has been suggested that cigarette smoking may affect IVF outcomes as well.

However, individual studies available on this issue do not always support a significant association between smoking and IVF success or oocyte quality. For example, in a study conducted in Australia in women undergoing IVF [6], the mean number of oocytes did not significantly differ between regular smokers (11.1, SD 6.5), ex-smokers (11.8, SD 10.1) or non-smokers (11.2, SD 7.6), indicating that smoking might not influence oocytes production. Interestingly, the same analysis showed that fertilization rates were not influenced by current smoking status but decreased as years of smoking increased ($p < 0.001$).

In a large Dutch nationwide retrospective analysis conducted on 8457 patients [42], no significant difference was observed in the mean number of oocytes retrieved between non-

smokers and smokers, yet there were significantly lower clinical pregnancy and live birth rates for smoking patients.

These findings regarding clinical pregnancies and live birth rate were further confirmed by more recent meta-analysis and reviews [8,9]. As regards to former smokers, they tend to have better ART outcomes than current smokers [7], but the evidence is scanty about the influence of smoking cessation on ART outcomes.

Vanegas et al. suggested that male smoking could also be associated with ART outcomes [43]. Whereas male current smoking did not affect the different stages of ART (egg retrieval, fertilization, embryo transfer, implantation, clinical pregnancy and livebirth), among past smokers every additional year since a man had quit smoking reduced the risk of failing ART by 4%, particularly between clinical pregnancy and live birth.

In our sample, good quality oocytes number was significantly lower in women who had smoked 20 or more cigarettes per day in the year before ART procedure, as compared to never smokers as well as to women currently smoking 5 or less cigarettes/day, and risk of embryo transfer failure was significantly higher than in never smokers. No significant effect was observed on clinical pregnancy and live birth. However, only six women had such a high level of smoking, thus our estimates should be considered with caution. Male smoking did not appear as a contributing factor to ART failure.

Leisure Physical Activity

Findings from literature are inconsistent. Among 2,232 patients prospectively enrolled before their first IVF cycle, Morris et al. [13] found that women who exercised 4 or more hours per week, for 1-9 years before ART cycle, were more likely to experience an implantation failure (OR 2.0, 95% CI 1.4-3.1) or pregnancy loss (OR 2.0, CI 1.2-3.4) than women who did not report exercise.

On the contrary, in a group of 131 women [14] those who were physically more active during the ART procedure were more likely to have an increased implantation rate and a live birth; none of these women met the criteria for high PA, so that the comparison was done between low and moderate PA. On the same line, an observational study [44] found that a self-reported active lifestyle in the preceding year affected favourably the ART outcome in 121 women, with clinical pregnancy more likely in women with a level above median for each kind of activity: active living (OR 1.96, 95% CI 1.09-3.50), sports/exercise (OR 1.48, 95% CI 1.02–2.15), and total activity (OR 1.52, 95% CI 1.15–2.01). Recently, findings from the EARTH Study [45] suggested that time spent in moderate-to-vigorous physical activities before IVF was not associated with probability of implantation, clinical pregnancy or live birth, in 273 women who underwent 427 IVF cycles. Pooled estimates from a recent meta-analysis [16] suggested a beneficial effect of PA on ART outcomes, and no effect on spontaneous abortion; despite this, study results are widely heterogeneous.

In our sample, an increased risk of embryo transfer failure was suggested in women with ≥5 hours/week of leisure PA, but this result was not significant in the multivariable analysis.

Strengths and Limitations.

Potential limitations should be considered. All information on smoking and drinking was self-reported by women, so some underestimates could have occurred. However, in Italy, alcohol consumption is socially accepted and recommendations to avoid alcohol to protect fertility have not received widespread attention and are not routinely advocated by gynaecologists before IVF.

Other sources of bias, including selection or confounding factors, are also unlikely to have produced marked effects, especially considering that all women were interviewed in the same institution and that participation was practically complete. Moreover, we analysed

information on nutritional status, and their inclusion into the model did not change the estimated RRs.

A further limitation was that knowledge regarding type of physical exercise was limited, because we recorded total number of weekly hours spent exercising, but not intensity or type of exercise.

Comparing the clinical pregnancy percentage in women who did not drink at all and those in the 3rd tertile of intake, the power of detecting a significant difference was about 13%. Using our data, with 30% prevalence of abstainers, we could identify a RR of not achieving clinical pregnancy of 1.8 for drinkers.

Lastly, this study only included women presenting for ART, thus the findings are not generalizable to the wider population.

Conclusions

Our study did not show an effect of alcohol consumption, current smoking and leisure physical activity on number of good quality oocytes and success rate after ART procedures.

Considering that reassuring results of our study were related to moderate alcohol intake and cigarette smoking, conservatively, all women seeking pregnancy should be advised to limit or avoid alcohol drinking and, of course, smoking. Moderate physical activity as a part of a healthy lifestyle is also advisable, although current knowledge does not support convincing evidence of a direct beneficial effect.

Declarations

Ethics approval and consent to participate.

The study protocol was approved by the Ethical Review Board of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano Area B (reference number 2616, 1421/2014).

All patients included gave their written informed consent to participate in the study.

Availability of data and materials.

The dataset analysed during the current study is available from the corresponding author on reasonable request.

Competing interests.

The authors declare that they do not have any competing interests.

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Data sharing.

Data are available upon reasonable request addressed to the corresponding author.

Authors' contribution

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

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Table 1. Demographic characteristics of 492 women, according to alcohol intake, smoking habits and leisure physical activity.

	Alcohol intake (g/day)								Smoking						Leisure Physical Activity					
	Abstainers 0		1st tertile 0.01-2.27		2nd tertile 2.28-5.74		3rd tertile ≥5.75		Never		Current		Former		< 2 hours/week		2-4 hours/week		≥5 hours/week	
	N=140	28.5%	N=117	23.8%	N=122	24.8%	N=113	23.0%	N=272	55.3%	N=90	18.3%	N=130	26.4%	N=256	54.1%	N=175	35.6%	N=51	10.4%
Age (years)																				
<35	**46**	**32.9**	**38**	**32.5**	**25**	**20.5**	**27**	**23.9**	71	26.1	28	31.1	38	29.2	78	29.3	47	26.9	11	21.6
35-39	**70**	**50.0**	**50**	**42.7**	**64**	**52.5**	**59**	**52.2**	131	48.2	46	51.1	65	50.0	123	46.2	89	50.9	31	60.8
≥40	**24**	**17.1**	**29**	**24.8**	**33**	**27.0**	**27**	**23.9**	70	25.7	16	17.8	27	20.8	65	24.4	39	22.3	9	17.6
College degree	**49**	**35.0**	**64**	**54.7**	**70**	**57.4**	**72**	**63.7**	**151**	**55.5**	**35**	**28.5**	**66**	**50.8**	**120**	**45.1**	**110**	**62.9**	**25**	**49.0**
Cause of infertility																				
Male factor only	37	26.4	35	29.9	33	27.0	23	20.4	**69**	**25.4**	**22**	**24.4**	**37**	**28.5**	68	25.6	47	26.9	13	25.5
Low ovarian reserve	26	18.6	25	21.4	24	19.7	22	19.5	**55**	**20.2**	**20**	**22.2**	**22**	**16.9**	51	19.2	36	20.6	10	19.6
Endometriosis	27	19.3	21	18.0	28	23.0	27	23.9	**52**	**19.1**	**19**	**21.1**	**32**	**24.6**	64	24.1	29	16.6	10	19.6
Ovulatory	10	7.1	5	4.3	4	3.3	0	0	**15**	**5.5**	**0**	**0**	**4**	**3.1**	12	4.5	5	2.9	2	3.9
Tubal	11	7.9	17	14.5	11	9.0	15	13.3	**19**	**7.0**	**16**	**17.8**	**19**	**14.6**	26	9.8	21	12.0	7	13.7
Unexplained	29	20.7	14	12.0	22	18.0	26	23.0	**62**	**22.8**	**13**	**14.4**	**16**	**12.3**	45	16.9	37	21.1	9	17.6
BMI (Kg/m^{-2})																				
<18.5	**12**	**8.6**	**11**	**9.4**	**12**	**9.8**	**12**	**10.6**	28	10.3	8	8.9	11	8.5	28	10.5	15	8.6	4	7.8
18.5-24.9	**98**	**70.0**	**80**	**68.4**	**97**	**79.5**	**94**	**83.2**	202	74.3	67	74.4	100	76.9	190	71.4	137	78.3	42	82.4
25.0-29.9	**16**	**11.4**	**15**	**12.8**	**8**	**6.6**	**7**	**6.2**	26	9.6	7	7.8	13	10.0	29	10.9	13	7.4	4	7.8
≥30.0	**14**	**10.0**	**11**	**9.4**	**5**	**4.1**	**0**	**0**	16	5.9	8	8.9	6	4.6	19	7.1	10	5.7	1	2.0
Occupational PA																				

Heavy/moderate	**50**	**35.7**	**27**	**23.3**	**33**	**27.1**	**28**	**24.8**	70	25.7	26	28.9	42	32.3	83	31.3	40	22.9	15	30.0
Mainly standing	**32**	**22.9**	**30**	**25.9**	**25**	**20.5**	**19**	**16.8**	58	21.3	22	24.4	26	20.0	60	22.6	32	18.3	14	28.0
Mainly sitting	**58**	**41.4**	**59**	**50.9**	**63**	**51.6**	**66**	**58.4**	144	52.9	42	46.7	60	46.2	122	46.1	103	58.9	21	42.0
Previous ART cycle	84	60.0	67	57.3	69	56.6	65	57.5	165	60.7	42	46.7	78	60.0	155	58.3	98	56.0	32	62.8
Mean calories (Kcal/day), mean (SD)	1711	(458)	1705	(401)	1782	(415)	1812	(493)	1764	(437)	1761	(446)	1712	(457)	**1788**	**(444)**	**1739**	**(425)**	**1597**	**(481)**
Mediterranean diet Score (n=473)*																				
0-4	51	38.4	37	32.5	33	28.0	34	31.5	75	28.6	32	37.2	25	20.0	**79**	**31.1**	**41**	**24.3**	**12**	**24.5**
5-6	53	39.8	52	45.6	53	44.9	45	41.7	113	43.1	31	36.1	56	44.8	**110**	**43.3**	**73**	**43.2**	**16**	**32.6**
7-9	29	21.8	25	21.9	32	27.1	29	26.8	74	28.2	23	26.7	44	35.2	**65**	**25.6**	**55**	**32.5**	**21**	**42.9**

Bold: $p<0.05$; sometimes the sums do not add up to the total because of missing values

ART: assisted reproduction technique; PA: physical activity; SD: standard deviation

*MDS without alcohol was calculated for class of alcohol intake

Table 2. Relative risks for failure in clinical outcomes of ART, in 492 women according to alcohol, smoking habits and leisure physical activity.

	N	Number of high-quality oocytes (median, Q1-Q3)	Missed Embryo transfer		ARR (95% CI)	Clinical pregnancy Failure		ARR (95% CI)	Live birth Failure		ARR (95% CI)
Alcohol intake			N	%		N	%		N	%	
Abstainers	140	5 (3-8)	16	11.4	1	92	65.7	1	102	72.9	1
1st tertile	117	4 (2-8)	14	12.0	1.08 (0.55-2.12)	72	61.5	0.95 (0.80-1.12)	84	71.8	1.00 (0.89-1.13)
2nd tertile	122	4 (3-7)	16	13.1	1.02 (0.53-1.96)	91	74.6	1.10 (0.93-1.29)	98	80.3	1.07 (0.95-1.21)
3rd tertile	113	5 (3-8)	19	16.8	1.32 (0.71-2.47)	80	70.8	1.05 (0.89-1.23)	87	77.0	1.05 (0.92-1.19)
≥1 drink/day	28	5 (3-8)	5	17.8	1.34 (0.53-3.40)	19	67.9	1.05 (0.78-1.41)	20	71.4	1.01 (0.76-1.34)
Current smoking											
Never	272	5 (3-8)	34	12.5	1	187	68.8	1	209	76.8	1
Current	91	5 (3-8)	13	14.4	1.42 (0.77-2.60)	64	71.1	1.08 (0.91-1.28)	68	75.6	1.03 (0.88-1.20)
Former	130	5 (3-8)	18	13.8	1.15 (0.68-1.94)	84	64.6	0.98 (0.86-1.11)	94	72.3	0.94 (0.84-1.08)
Current ≤ 5 cig/day	42	5 (3-7)	8	19.0	1.70 (0.84-3.43)	32	74.4	1.11 (0.88-1.41)	34	79.1	1.06 (0.88-1.27)
Current > 5 cig/day	48	4 (3-8)	5	10.4	1.13 (0.46-2.80)	32	68.1	1.04 (0.84-1.29)	34	72.3	0.98 (0.83-1.16)
Current ≥20 cig/day	6	3 (2-6)*	2	33.3	5.20 (1.40-19.2)	5	83.3	1.00 (0.69-1.73)	5	83.3	1.08 (0.65-1.79)

Former (1-5 years)	75	5 (3-9)	14	18.7	1.62 (0.92-2.84)	51	68.0	1.02 (0.87-1.19)	56	74.7	0.99 (0.87-1.12)
Former (>5 years)	55	5 (2-9)**	4	7.3	0.57 (0.21-1.54)	33	60.0	0.92 (0.76-1.12)	38	69.1	0.94 (0.82-1.09)
Leisure PA											
<2 h/wk	266	5 (3-8)	35	13.2	1	179	67.3	1	199	74.8	1
2-4	175	4 (3-8)	20	11.4	0.83 (0.48-1.43)	113	64.6	0.99 (0.90-1.08)	128	73.1	0.99 (0.91-1.09)
≥5	51	4 (2-8)	10	19.6	1.75 (0.91-3.40)	43	84.3	1.11 (0.96-1.28)	44	86.3	1.10 (0.95-1.28)

ARR: adjusted relative risk; CI: confidence interval; PA: physical activity; the final model included age class, college degree, BMI class (<25.0, ≥25.0), occupational PA, previous ART cycles, calories intake. Cause for infertility for smoking habits, and MDS score for PA were also included.

* p=0.047 as compared to never smokers

** p=0.003 as compared to never smokers

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title and abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	p.2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	p. 4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	p.5
Methods			
Study design	4	Present key elements of study design early in the paper	p. 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	p. 5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	p. 6-7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	p. 6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	p. 6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	p. 6-7
Bias	9	Describe any efforts to address potential sources of bias	p.8
Study size	10	Explain how the study size was arrived at	p. 8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	p.8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	p.8
		(*b*) Describe any methods used to examine subgroups and interactions	p.8
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods	-

		taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	No sensitivity analyses

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	p.9
		(b) Give reasons for non-participation at each stage	n.a.
		(c) Consider use of a flow diagram	n.a.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	p.9
		(b) Indicate number of participants with missing data for each variable of interest	p.9
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) *Implicit: for each women, the time between ART initiation and outcome (embryo transfer, clinical pregnancy and livebirth)*	-
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*p.9*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	p.9-10 Table 1 and 2
		(*b*) Report category boundaries when continuous variables were categorized	p.9-10 Table 1 and 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	p. 9
Discussion			
Key results	18	Summarise key results with reference to study objectives	p.11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p.14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	p.11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	p.14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	p. 16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Secondary Subject Heading:	Smoking and tobacco, Sports and exercise medicine
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Pre-treatment maternal lifestyle and outcomes of assisted reproduction: an Italian cohort study.

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Abstract

Objective. We investigated whether lifestyle affects assisted reproduction technique (ART) outcomes.

Design. Cohort study.

Setting. Italian Fertility Unit.

Participants. From September 2014 to December 2016, women from couples presenting for evaluation and eligible for ART were invited to participate. Information on alcohol intake, current smoking and leisure physical activity during the year before the interview was collected, using a structured questionnaire. We considered the ART outcomes of the cycle immediately following the interview.

Primary and secondary outcome measures. The primary outcome measure was cumulative pregnancy rate per retrieval. Secondary measures were number of retrieved oocytes, embryo transfer and live birth.

Results. In 492 women undergoing an ART cycle, 427 (86.8%) underwent embryo transfer, 157 (31.9%) had at least one clinical pregnancy, 121 (24.6%) had live birth. The cumulative pregnancy rate per retrieval was 33.3% (95% CI 28.5%-38.7%). In women in the 3rd tertile of alcohol intake, adjusted relative risk (ARR) was 0.97 (95% confidence interval (CI) 0.87-1.08), 0.90 (95% CI 0.62-1.30) and 0.89 (95% CI 0.57-1.37) for embryo transfer, clinical pregnancy and live birth respectively. The corresponding figures in women currently smoking more than 5 cigarettes/day were 1.00 (95% CI 0.88-1.16), 0.94 (95% CI 0.60-1.48), and 1.14 (95% CI 0.68-1.90), and in women with physical activity ≥5 hours/week were 0.93 (95% CI 0.79-1.08), 0.44 (95% CI 0.22-0.90), and 0.48 (95% CI 0.22-1.05) respectively.

Conclusion. There were no significant differences in IVF outcomes among women who used alcohol or tobacco in the year prior to treatment. Conservatively, all women should be advised

to limit substance abuse. Moreover, our study suggested that maintaining a moderate, but not high, level of physical activity could be beneficial.

Keywords. Alcohol intake, smoking habits, leisure physical activity, assisted reproduction techniques, pregnancy, live birth.

Strengths and limitations of this study

- This study analysed several lifestyle factors of women interviewed in the same institution, participation was practically complete, and information on nutritional status was also available.
- Smoking and drinking habits were self-reported by women, so some underestimates could have occurred.
- Information on type of physical exercise was limited, because total number of weekly hours spent exercising was recorded, but not intensity or type of exercise.
- These findings regard women presenting for ART and are not generalizable to the fertile population.

Introduction

Alcohol consumption and smoking are among the most common lifestyle exposures in women. During the last decades, the relationship between these lifestyle factors and spontaneous fertility has been investigated in several observational studies: some have shown that alcohol and smoking affect spontaneous fertility [1,2], although not consistently [3,4].

These exposures may contribute to spontaneous reproductive failures, but they may also impair the success rate of assisted reproduction technology (ART). Thus, it is conceivable that modifying such lifestyle habits before treatments could reduce the need for ART procedures and/or enhance the likelihood of in vitro fertilization (IVF) success. In a study conducted in California, alcohol intake was negatively associated with the number of oocytes retrieved, but not with live birth rate [5]. Otherwise, in a prospective cohort study, no association emerged between alcohol intake during days 4–10 of ovarian stimulation and IVF outcomes [6]. A recent systematic revision of literature confirmed that average alcohol intake before ART initiation did not impact on the outcomes, whereas intake at the start of ART cycle had a negative effect on fertilization, embryo quality and implantation [7].

Cigarette smoking is the most common lifestyle factor that could affect IVF outcomes and several studies have demonstrated the negative effect of smoking on pregnancy rate and upon clinical outcome of ART [8]. However, literature on this issue is still limited [9], although a recent meta-analysis [10] found that current smokers undergoing ART had lower clinical pregnancy and live birth rate than non-smokers and experienced a significant increase of spontaneous miscarriage.

Evidence on physical activity (PA) was inconsistent: although data from the Nurses' Health Study II suggested that vigorous activity may reduce ovulatory infertility [11], a Norwegian cohort indicated that high intensity and frequency of PA increase sub-fertility [12]. Studies specifically on PA and ART success were equally inconsistent: investigating pre-treatment

PA, Morris et al. [13] found that women undergoing ART had a 40% reduced likelihood of live birth, if engaged in PA 4 hours or more per week for less than 10 years, compared with women not regularly engaged in PA. Another study [14] did not find a beneficial effect of activity levels before treatment on clinical outcomes, although moderate PA during ART cycle was associated with higher implantation and live birth rates. Recently, it was suggested that health-promoting lifestyle education may increase the success rates of ART, correcting risk factors that negatively affect fertility [15]. This evidence has been recently reviewed: pooled estimates from a systematic research found that physical activity before ART cycles was associated with increased rates of clinical pregnancy and live births, but no effect was shown on miscarriage rate [16].

These factors have been analysed in relation to sperm quality. Several reviews showed that the same lifestyle habits, such as smoking and regular drinking, negatively impacting female fertility, also have a detrimental effect on semen quality [17,18]. On the contrary, the effect of physical activity is still under discussion [19,20].

Alcohol consumption [21], smoking habits [22] and physical activity [23,24] largely vary in different populations. Thus, it is interesting to analyse the role of these lifestyles on fertility treatment in an Italian setting, using data from a cohort study conducted in an Italian Fertility Centre.

Methods

From September 2014 to December 2016, in randomly selected days, sub-fertile couples, presenting for evaluation to the Fertility Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore, Policlinico, Milan, and eligible for ART, were invited to participate into a cohort study on the role of lifestyle habits and diet on ART outcomes. The study protocol was

approved by the Institutional Ethical 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. The time interval between the proposal of the study and the interview was generally less than one month.

The overall participation rate was close to 95%, mainly since couples were interviewed during the period spent waiting for the different diagnostic stages, before actual ART procedures, and the not sensitive character of questions.

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, anthropometric variables, personal medical history and reproductive history, and lifestyle factors. Couples that cannot speak Italian were excluded from the study.

Patient and public involvement.

Patients were not involved in the design, recruitment or conduct of the study.

The present study reported on the outcome of the cycle immediately following the interview.

Procedures

To evaluate the effect of recent exposure, patients were asked to report about their usual weekly food consumption in the last year, using a reproducible and valid food frequency questionnaire [25–27], including the weekly numbers of drinks for several alcoholic beverages. The questionnaire was satisfactorily reproducible [28]. Taking into account the different ethanol concentration, one unit corresponded to approximately 125 ml of wine, 330 ml of beer and 30 ml of hard liquor (i.e., about 12.5 g of ethanol). Total alcohol intake,

expressed in grams of ethanol per day (g/day), was computed as the sum of all reported alcoholic beverages. “Never drinkers” and “Ex- drinkers” were patients who abstained from drinking lifelong and for at least 12 months at the time of interview, respectively. For the purpose of this study, we considered these two groups of women in the same category “Abstainers”.

A woman was considered a smoker if she had smoked ≥ one cigarette/day for at least one year; a former smoker if she had smoked ≥ one cigarette/day for at least one year, but had stopped more than one year before the interview, and a non-smoker if she had never smoked ≥ one cigarette/day.

Before starting ovarian stimulation, women were advised to abstain from alcohol and smoking, thus no such exposure should occur during ART cycle.

The adherence to the Mediterranean diet was assessed through an a priori score (Mediterranean diet score, MDS), developed by Trichopoulou and colleagues [29] and calculated as previously published [30]. To include MDS score in the analysis of alcohol intake, it was recalculated excluding alcohol. Satisfactory reproducibility of questions on self-reported smoking and drinking habits in our study populations has been previously reported [31].

Leisure PA was defined as the number of hours per week of a sport or activity such as walking, gardening, cycling, etc., in the year preceding the interview. Scores ranged between 1 and 4, corresponding to <2, 2–4, 5-7 and >7 h of PA per week. No information was available on intensity of activity.

Patients were managed according to a standardized clinical protocol as reported in details elsewhere [32,33]. The choice between conventional IVF or Intra-Cytoplasmatic Sperm Injection (ICSI) was made based on semen characteristics. Good quality oocytes were those in

metaphase I-II for IVF and metaphase II for ICSI. The main outcome was the cumulative pregnancy rate per retrieval in the cycle immediately following the interview.

All clinical information (including infertility diagnosis) was collected from medical records.

Statistical analysis

Clinical pregnancy was considered the main objective of the study. Considering a 30% of pregnancy rate per cycle, as usual in our Fertility Centre, this study was powered to detect a 1.5 increase of risk in the highest tertile of intake as compared to the lowest (α=0.05, β=0.80). Multiple outcomes were considered: 1. Number of retrieved good quality oocytes; 2. Embryo transfer; 3. Clinical pregnancy; 4: Live birth. Patients who failed each treatment stage were included in the following stage as failures. Women with a previous miscarriage and a pregnancy or livebirth after the following embryo transfer were considered as having a successful pregnancy and livebirth.

Categorical variables were described as frequency (N) and percentage (%) and compared using the Pearson or Mantel-Haenzsel chi-square, as appropriate. Continuous variables were described as mean and standard deviation (SD) if normally distributed, or median and interquartile range (IQR) if not normally distributed and analysed using analysis of variance and Kruskal-Wallis test respectively. Correlations were evaluated using Pearson r or Spearman rho coefficients, as appropriate.

We used multivariable generalized linear mixed models to evaluate the association of exposure variables with treatment outcomes. We used a Poisson distribution and log link function for the number of good quality oocytes retrieved, and binomial distribution and logit link function for clinical outcomes. We estimated relative risks (RRs) of each clinical outcome and corresponding 95% CIs in categories of alcohol intake (approximate tertiles), former and current smoking (no, ≤ 5, >5 cigarettes/day) and leisure PA (<2, 2-4, ≥5 hours/week) in the year before the interview.

To account for potential confounders, we included terms for variables, that were associated with these modifiable lifestyles, and/or with at least one ART outcome, in the general linear model and multiple log-binomial regression models (as indicated in table footnotes). Terms for interaction were tested.

All the analyses were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

From September 2014 to December 2016, out of 501 women undergoing ART cycle, 9 (1.8%) did not provide complete information about their lifestyle, or were lost to follow-up, and were excluded from this analysis. Analysis was then performed on 492 ART cycle outcomes from 492 women.

Mean age was 36.6 years (standard deviation, SD, 3.6 range 27-45) and mean body mass index (BMI) was 22.3 kg/m^2 (SD 3.9, range 16.4-41.7). Thirty women (6.1%) were obese (BMI $\geq$ 30.0 kg/m^2).

The characteristics of women according to alcohol, smoking habits and PA are shown in Table 1.

Only 16 women (3.2%) exercised more than 7 hours per week, so we merged the two categories 5-7 and >7 hours/week. Leisure PA was associated with college degree and higher MDS, and inversely with daily calories intake.

Of the 492 initiated cycles in each woman, 427 (86.8%) resulted in embryo transfer, 157 (31.9%) in clinical pregnancy, 121 (24.6%) in live births. Out of 36 clinical pregnancies not resulting in live birth, 34 ended with miscarriage, one with an induced abortion, and one was extra-uterine.

Seventy-two (14.6%) women underwent two, 28 (5.7%) three, 4 (0.8%) ≥4 embryo transfers. Out of 157 women with pregnancy, 7 had a miscarriage at first attempt and livebirth at the second one, for a total of 164 pregnancies in 492 women. The cumulative pregnancy rate per retrieval was 33.3% (95% CI 28.5%-38.7%).

Age was the main risk factor for ART failure. The median of good quality oocytes was 6 (IQR 4-9) in women <35 years old, 5 (IQR 3-8) in 35-39, 3 (IQR 2-6) in women aged ≥ 40 years (p<0.0001). No association was observed at univariate analysis with alcohol intake, current smoking or leisure PA. As compared to women aged < 35, RR for successful embryo transfer was 0.92 (95% CI 0.86-0.98) for women aged 35-39 and 0.90 (95% CI 0.82-0.98) for those aged ≥40 years. The corresponding figures were 0.78 (95% CI 0.59-1.01) and 0.44 (95% CI 0.29-0.68) for clinical pregnancy; 0.66 (95% CI 0.48-0.91) and 0.34 (95% CI 0.20-0.59) for live birth.

At univariate analysis, leisure PA ≥ 5 hours/week was significantly associated with lower risk of clinical pregnancy (RR 0.48, 95% CI 0.25-0.93), whereas no relationship was observed with embryo transfer and live birth. Alcohol intake and current smoking were not significantly associated with any ART outcomes.

Table 2 shows the relation between exposures and clinical results, accounting for potential confounders. No significant association was observed between smoking, alcohol intake and leisure PA, thus they were not mutually adjusted. Terms for interaction between smoking, alcohol intake and leisure PA did not show any significance and were excluded from the final models.

In this sample, 28 (5.7%) women drank at least 1 alcohol unit per day. Although the ARR for embryo transfer was lower, it was not significant as compared to abstainers, and no effect was observed on other outcomes. ARR for embryo transfer was significantly lower in six women who smoked ≥20 cigarettes/day during the year before ART procedure. Former smokers who

stopped smoking more than 5 years before undergoing ART had a higher number of oocytes than never smokers.

Analysing alcohol intake (g) and number of cigarettes as continuous variables, we did not find any significant correlation with number of high-quality oocytes. Since most women did not smoke, median intakes were 0 (IQR 0-0) in all categories and no differences could be observed. As regards alcohol, women who underwent embryo transfer had median intakes lower than those who did not (1.9 (IQR 0-5.3) vs. 2.7 (IQR 0-7.5 g/day), p=0.16); those who achieved clinical pregnancy consumed 1.8 (IQR 0-4.7) g/day and those who did not achieve it consumed 2.4 (IQR 0-5.6) g/day (p=0.11). Women with livebirth had lower alcohol intake than those without livebirth (1.8 (IQR 0-5.3) vs 2.3 (IQR 0-5.6) g/day, p=0.20). None of these differences was statistically significant.

Considering ICSI and IVF separately, ART outcomes were similar, and including this variable in the equations did not affect the risk estimation.

Finally, we controlled these results for partner's characteristics and lifestyle, in a subgroup of 324 couples with complete information for both male and female. Men's age was significantly associated with higher rate of negative outcomes in the univariate analysis, but when including women's age in the model this relationship lost significance. As regards to women lifestyle, results did not change. Men's lifestyle (smoking, alcohol drinking and physical activity) did not significantly impact on ART outcomes.

Discussion

In this sample of women referring to an Italian Fertility centre, did not play a significant role in the outcome of ART, except for heavy smoking, associated with fewer good quality oocytes, and high physical activity, associated with lower rate of pregnancy

Alcohol

The role of alcohol intake on spontaneous fertility has been associated with decreased fertility and chance of conception [4,34,35]. A recent meta-analysis of observational studies, including about 100,000 women, suggested that alcohol consumption was associated with reduced fecundability [36]. In biological terms, alcohol may lower fertility affecting endogenous hormone levels [37] and embryo quality [38].

The role of alcohol intake on ART success rate has been analysed in some studies showing inconsistent results [5,6,39,40]. These differences may be partially due to the high heterogeneity between studies, in particular in terms of intake prevalence. For example, in Boston, US, a study, found that 30% of women reporting >1 unit/day of alcohol intake had an increased risk of adverse outcome, but no association between low to moderate alcohol consumption was observed [39]. Literature suggests that alcohol drinking at start of ART may exert a detrimental effect, but the evidence is still limited [7].

Along this line, we did not find any association between alcohol intake and ART outcomes, but we were not able to analyse the effect of high alcohol intake, present in only 5.7% of our sample.

Smoking

Since it appears to have a detrimental effect on spontaneous fertility [1,41], it has been suggested that cigarette smoking may affect IVF outcomes as well.

However, individual studies available on this issue do not always support a significant association between smoking and IVF success or oocyte quality. For example, in a study conducted in Australia in women undergoing IVF [6], the mean number of oocytes did not significantly differ between regular smokers (11.1, SD 6.5), ex-smokers (11.8, SD 10.1) or non-smokers (11.2, SD 7.6), indicating that smoking might not influence oocytes production. Interestingly, the same analysis showed that fertilization rates were not influenced by current smoking status but decreased as years of smoking increased ($p < 0.001$).

In a large Dutch nationwide retrospective analysis conducted on 8457 patients [42], no significant difference was observed in the mean number of oocytes retrieved between non-smokers and smokers, yet there were significantly lower clinical pregnancy and live birth rates for smoking patients.

These findings regarding clinical pregnancies and live birth rate were further confirmed by more recent meta-analysis and reviews [8,9]. As regards to former smokers, they tend to have better ART outcomes than current smokers [7], but the evidence is scanty about the influence of smoking cessation on ART outcomes.

Vanegas et al. suggested that male smoking could also be associated with ART outcomes [43]. Whereas male current smoking did not affect the different stages of ART (egg retrieval, fertilization, embryo transfer, implantation, clinical pregnancy and livebirth), among past smokers every additional year since a man had quit smoking reduced the risk of failing ART by 4%, particularly between clinical pregnancy and live birth.

In our sample, good quality oocytes number was significantly lower in women who had smoked 20 or more cigarettes per day in the year before ART procedure, as compared to never smokers, as well as to women currently smoking 5 or less cigarettes/day. No significant effect was observed on clinical pregnancy and live birth. However, only six women had such a high level of smoking, thus our estimates should be considered with caution. Male smoking did not appear as a contributing factor to ART failure.

Leisure Physical Activity

Findings from literature are inconsistent. Among 2,232 patients prospectively enrolled before their first IVF cycle, Morris et al. [13] found that women who exercised 4 or more hours per week, for 1-9 years before ART cycle, were more likely to experience an implantation failure

(OR 2.0, 95% CI 1.4-3.1) or pregnancy loss (OR 2.0, CI 1.2-3.4) than women who did not report exercise.

On the contrary, in a group of 131 women [14] those who were physically more active during the ART procedure were more likely to have an increased implantation rate and a live birth; none of these women met the criteria for high PA, so that the comparison was done between low and moderate PA. On the same line, an observational study [44] found that a self-reported active lifestyle in the preceding year affected favourably the ART outcome in 121 women, with clinical pregnancy more likely in women with a level above median for each kind of activity: active living (OR 1.96, 95% CI 1.09-3.50), sports/exercise (OR 1.48, 95% CI 1.02–2.15), and total activity (OR 1.52, 95% CI 1.15–2.01). Recently, findings from the EARTH Study [45] suggested that time spent in moderate-to-vigorous physical activities before IVF was not associated with probability of implantation, clinical pregnancy or live birth, in 273 women who underwent 427 IVF cycles. Pooled estimates from a recent meta-analysis [16] suggested a beneficial effect of PA on ART outcomes, and no effect on spontaneous abortion; despite this, study results are widely heterogeneous.

In our sample, we found a lower risk of clinical pregnancy in women with ≥5 hours/week of leisure PA, but this relationship was not significant as regards livebirth.

Strengths and Limitations.

Potential limitations should be considered. All information on smoking and drinking was self-reported by women, so some underestimates could have occurred. However, in Italy, alcohol consumption is socially accepted and recommendations to avoid alcohol to protect fertility have not received widespread attention and are not routinely advocated by gynaecologists before IVF.

Other sources of bias, including selection or confounding factors, are also unlikely to have produced marked effects, especially considering that all women were interviewed in the same

institution and that participation was practically complete. Moreover, we analysed information on nutritional status, and their inclusion into the model did not change the estimated RRs.

A further limitation was that knowledge regarding type of physical exercise was limited, because we recorded total number of weekly hours spent exercising, but not intensity or type of exercise.

Comparing the clinical pregnancy percentage in women who did not drink at all and those in the 3rd tertile of intake, the power of detecting a significant difference was about 13%. Using our data, with 30% prevalence of abstainers, we could identify a RR of not achieving clinical pregnancy of 1.8 for drinkers.

Lastly, this study only included women presenting for ART, thus the findings are not generalizable to the wider population.

Conclusions

Our study did not show significant differences in IVF outcomes among women who used alcohol or tobacco in the year prior to treatment. Considering that reassuring results of our study were related to moderate alcohol intake and cigarette smoking, conservatively all women seeking pregnancy should be advised to limit or avoid substance abuse. Moderate physical activity as a part of a healthy lifestyle is also advisable, although current knowledge does not support consistent evidence of a direct beneficial effect.

Declarations

Ethics approval and consent to participate.

The study protocol was approved by the Ethical Review Board of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano Area B (reference number 2616, 1421/2014).

All patients included gave their written informed consent to participate in the study.

Availability of data and materials.

The dataset analysed during the current study is available from the corresponding author on reasonable request.

Competing interests.

The authors declare that they do not have any competing interests.

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Data sharing.

Data are available upon reasonable request addressed to the corresponding author.

Authors' contribution

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

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Table 1. Demographic characteristics of 492 women, according to alcohol intake, smoking habits and leisure physical activity.

	Alcohol intake (g/day)								Smoking						Leisure Physical Activity					
	Abstainers 0		1st tertile 0.01-2.27		2nd tertile 2.28-5.74		3rd tertile ≥5.75		Never		Current		Former		< 2 hours/week		2-4 hours/week		≥5 hours/week	
	N=140	28.5%	N=117	23.8%	N=122	24.8%	N=113	23.0%	N=272	55.3%	N=90	18.3%	N=130	26.4%	N=256	54.1%	N=175	35.6%	N=51	10.4%
Age (years)																				
<35	**46**	**32.9**	**38**	**32.5**	**25**	**20.5**	**27**	**23.9**	71	26.1	28	31.1	38	29.2	78	29.3	47	26.9	11	21.6
35-39	**70**	**50.0**	**50**	**42.7**	**64**	**52.5**	**59**	**52.2**	131	48.2	46	51.1	65	50.0	123	46.2	89	50.9	31	60.8
≥40	**24**	**17.1**	**29**	**24.8**	**33**	**27.0**	**27**	**23.9**	70	25.7	16	17.8	27	20.8	65	24.4	39	22.3	9	17.6
College degree	**49**	**35.0**	**64**	**54.7**	**70**	**57.4**	**72**	**63.7**	**151**	**55.5**	**35**	**28.5**	**66**	**50.8**	**120**	**45.1**	**110**	**62.9**	**25**	**49.0**
Cause of infertility																				
Male factor only	37	26.4	35	29.9	33	27.0	23	20.4	**69**	**25.4**	**22**	**24.4**	**37**	**28.5**	68	25.6	47	26.9	13	25.5
Low ovarian reserve	26	18.6	25	21.4	24	19.7	22	19.5	**55**	**20.2**	**20**	**22.2**	**22**	**16.9**	51	19.2	36	20.6	10	19.6
Endometriosis	27	19.3	21	18.0	28	23.0	27	23.9	**52**	**19.1**	**19**	**21.1**	**32**	**24.6**	64	24.1	29	16.6	10	19.6
Ovulatory	10	7.1	5	4.3	4	3.3	0	0	**15**	**5.5**	**0**	**0**	**4**	**3.1**	12	4.5	5	2.9	2	3.9
Tubal	11	7.9	17	14.5	11	9.0	15	13.3	**19**	**7.0**	**16**	**17.8**	**19**	**14.6**	26	9.8	21	12.0	7	13.7
Unexplained	29	20.7	14	12.0	22	18.0	26	23.0	**62**	**22.8**	**13**	**14.4**	**16**	**12.3**	45	16.9	37	21.1	9	17.6
BMI (Kg/m^{-2})																				
<18.5	**12**	**8.6**	**11**	**9.4**	**12**	**9.8**	**12**	**10.6**	28	10.3	8	8.9	11	8.5	28	10.5	15	8.6	4	7.8
18.5-24.9	**98**	**70.0**	**80**	**68.4**	**97**	**79.5**	**94**	**83.2**	202	74.3	67	74.4	100	76.9	190	71.4	137	78.3	42	82.4
25.0-29.9	**16**	**11.4**	**15**	**12.8**	**8**	**6.6**	**7**	**6.2**	26	9.6	7	7.8	13	10.0	29	10.9	13	7.4	4	7.8
≥30.0	**14**	**10.0**	**11**	**9.4**	**5**	**4.1**	**0**	**0**	16	5.9	8	8.9	6	4.6	19	7.1	10	5.7	1	2.0
Occupational PA																				

Heavy/moderate	**50**	**35.7**	**27**	**23.3**	**33**	**27.1**	**28**	**24.8**	70	25.7	26	28.9	42	32.3	83	31.3	40	22.9	15	30.0
Mainly standing	**32**	**22.9**	**30**	**25.9**	**25**	**20.5**	**19**	**16.8**	58	21.3	22	24.4	26	20.0	60	22.6	32	18.3	14	28.0
Mainly sitting	**58**	**41.4**	**59**	**50.9**	**63**	**51.6**	**66**	**58.4**	144	52.9	42	46.7	60	46.2	122	46.1	103	58.9	21	42.0
Previous ART cycle	84	60.0	67	57.3	69	56.6	65	57.5	165	60.7	42	46.7	78	60.0	155	58.3	98	56.0	32	62.8
Mean calories (Kcal/day), mean (SD)	1711	(458)	1705	(401)	1782	(415)	1812	(493)	1764	(437)	1761	(446)	1712	(457)	**1788**	**(444)**	**1739**	**(425)**	**1597**	**(481)**
Mediterranean diet Score (n=473)*																				
0-4	51	38.4	37	32.5	33	28.0	34	31.5	75	28.6	32	37.2	25	20.0	**79**	**31.1**	**41**	**24.3**	**12**	**24.5**
5-6	53	39.8	52	45.6	53	44.9	45	41.7	113	43.1	31	36.1	56	44.8	**110**	**43.3**	**73**	**43.2**	**16**	**32.6**
7-9	29	21.8	25	21.9	32	27.1	29	26.8	74	28.2	23	26.7	44	35.2	**65**	**25.6**	**55**	**32.5**	**21**	**42.9**

Bold: $p<0.05$; sometimes the sums do not add up to the total because of missing values

ART: assisted reproduction technique; PA: physical activity; SD: standard deviation

*MDS without alcohol was calculated for class of alcohol intake

Table 2. Relative risks for clinical outcomes of ART, in 492 women according to alcohol, smoking habits and leisure physical activity.

	N	Number of high-quality oocytes (median, Q1-Q3)	Embryo transfer		ARR (95% CI)	Clinical pregnancy		ARR (95% CI)	Live birth		ARR (95% CI)
Alcohol intake			N	%		N	%		N	%	
Abstainers	140	5 (3-8)	16	11.4	1	92	65.7	1	102	72.9	1
1st tertile	117	4 (2-8)	14	12.0	0.99 (0.90-1.10)	72	61.5	1.11 (0.81-1.53)	84	71.8	1.01 (0.68-1.51)
2nd tertile	122	4 (3-7)	16	13.1	0.99 (0.89-1.09)	91	74.6	0.80 (0.54-1.18)	98	80.3	0.77 (0.48-1.21)
3rd tertile	113	5 (3-8)	19	16.8	0.97 (0.87-1.08)	80	70.8	0.90 (0.62-1.30)	87	77.0	0.89 (0.57-1.37)
≥1 drink/day	28	5 (3-8)	5	17.8	0.97 (0.81-1.18)	19	67.9	1.12 (0.62-2.00)	20	71.4	1.31 (0.69-2.48)
Current smoking											
Never	272	5 (3-8)	34	12.5	1	187	68.8	1	209	76.8	1
Current	91	5 (3-8)	13	14.4	0.98 (0.88-1.09)	64	71.1	0.91 (0.62-1.33)	68	75.6	1.09 (0.70-1.69)
Former	130	5 (3-8)	18	13.8	0.99 (0.92-1.09)	84	64.6	1.08 (0.81-1.45)	94	72.3	1.13 (0.80-1.60)
Current ≤ 5 cig/day	42	5 (3-7)	8	19.0	0.95 (0.92-1.10)	32	74.4	0.82 (0.49-1.39)	34	79.1	0.92 (0.50-1.68)
Current > 5 cig/day	48	4 (3-8)	5	10.4	1.00 (0.88-1.16)	32	68.1	0.94 (0.60-1.48)	34	72.3	1.14 (0.68-1.90)
Current ≥20 cig/day	6	3 (2-6)*	2	33.3	0.84 (0.54-1.32)	5	83.3	0.43 (0.07-2.55)	5	83.3	0.61 (0.10-3.67)

Former (1-5 years)	75	5 (3-9)	14	18.7	0.96 (0.86-1.07)	51	68.0	0.99 (0.68-1.44)	56	74.7	1.05 (0.68-1.61)
Former (>5 years)	55	5 (2-9)**	4	7.3	1.03 (0.88-1.21)	33	60.0	1.22 (0.84-1.66)	38	69.1	1.28 (0.81-2.01)
Leisure PA											
<2 h/wk	266	5 (3-8)	35	13.2	1	179	67.3	1	199	74.8	1
2-4	175	4 (3-8)	20	11.4	1.02 (0.94-1.11)	113	64.6	1.13 (0.86-1.48)	128	73.1	1.08 (0.68-1.50)
≥5	51	4 (2-8)	10	19.6	0.93 (0.79-1.08)	43	84.3	0.44 (0.22-0.90)	44	86.3	0.48 (0.22-1.05)

ARR: adjusted relative risk; CI: confidence interval; PA: physical activity; the final model included age class, college degree, BMI class (<25.0, ≥25.0), occupational PA, previous ART cycles, calories intake. Cause for infertility for smoking habits, and MDS score for PA were also included.

* p=0.047 as compared to never smokers

** p=0.003 as compared to never smokers

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title and abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	p.2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	p. 4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	p.5
Methods			
Study design	4	Present key elements of study design early in the paper	p. 5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	p. 5-6
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	p. 6-7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	p. 6-7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	p. 6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	p. 6-7
Bias	9	Describe any efforts to address potential sources of bias	p.8
Study size	10	Explain how the study size was arrived at	p. 8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	p.8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	p.8
		(*b*) Describe any methods used to examine subgroups and interactions	p.8
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods	-

		taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	No sensitivity analyses

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	p.9
		(b) Give reasons for non-participation at each stage	n.a.
		(c) Consider use of a flow diagram	n.a.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	p.9
		(b) Indicate number of participants with missing data for each variable of interest	p.9
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) *Implicit: for each women, the time between ART initiation and outcome (embryo transfer, clinical pregnancy and livebirth)*	-
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*p.9*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	-
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	p.9-10 Table 1 and 2
		(*b*) Report category boundaries when continuous variables were categorized	p.9-10 Table 1 and 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	p. 9
Discussion			
Key results	18	Summarise key results with reference to study objectives	p.11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	p.14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	p.11-13
Generalisability	21	Discuss the generalisability (external validity) of the study results	p.14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	p. 16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.