

RESEARCH ARTICLE

Investigating the effect of lifestyle risk factors upon number of aspirated and mature oocytes in in vitro fertilization cycles: Interaction with antral follicle count

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Abstract

Introduction

There is evidence demonstrating that certain lifestyle factors have a detrimental effect on fertility. Since such factors often coexist, possible synergistic effects merit further investigation. Thus we aimed to examine the cumulative impact of lifestyle factors on in vitro fertilization (IVF) early reproductive treatment outcomes and their interaction with measures of ovarian reserve.

Materials and methods

By following women who were starting their first fresh IVF cycle in 2 cohorts, the "Lifestyle study cohort" (hypothesis generating cohort, n = 242) and the "UppSTART study" (validation cohort, n = 432) in Sweden, we identified two significant risk factors acting independently, smoking and BMI, and then further assessed their cumulative effects.

Results

Women with both these risk factors had an Incidence Rate Ratio (IRR) of 0.75 [(95% CI 0.61–0.94)] regarding the number of aspirated oocytes compared to women without these risk factors. Concerning the proportion of mature oocytes in relation to the total number of aspirated oocytes, the interaction between BMI and Antral Follicle Count (AFC) was significant (p-value 0.045): the lower the value of AFC, the more harmful the effect of BMI with the outcome.

responsible departments upon reasonable request. Any interested authors are welcome to contact the authors, who will then fill out the agreements necessary when sharing data and after approval by the Regional Ethics Board in Stockholm and Uppsala. All data regarding the current study and "Lifestyle study" are available on request to the Department of Women's and Children's Health, Uppsala University; kbh@kbh.uu.se. Additional data regarding "UppStART study" are available on request to the Department of Medical Epidemiology and Biostatistics, Karolinska Institutet; <https://ki.se/meb/uppstart>.

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Conclusions

Data shows that there is an individual as well as a cumulative effect of smoking and BMI on the number of aspirated and mature oocytes in fresh IVF treatment cycles. AFC might modify associations between BMI and the proportion of mature oocytes in relation to the total number of aspirated oocytes. These results highlight the importance of lifestyle factors on IVF early reproductive outcomes and provide additional evidence for the importance of pre-conception guidance for the optimization of IVF cycle outcome.

Introduction

Infertility is a serious concern for individuals wishing to start a family and can be costly for both the couple and for society. Approximately 10–15% of those who try to conceive suffer from infertility and often turn to medically assisted reproduction techniques [1]. Contrary to the perception of many, undergoing In Vitro Fertilization (IVF) does not guarantee success; 38–69% of couples who begin IVF will remain childless, even if they undergo as many as six IVF cycles [2]. This underlies the importance of identification of potentially modifiable predictors of successful treatment. Lifestyle factors including obesity, smoking, stress, and most importantly, postponing parenthood until an advanced age, have been shown to contribute to reduced fertility [3].

The relevance of personal behavior and lifestyle-related factors that may adversely affect fertility and ART outcomes has increasingly been discussed over the last ten years [4,5]. Reviewing the impact of certain lifestyle habits on IVF outcomes can motivate patients to modify behaviors such as smoking, heavy alcohol consumption or excessive body weight, since changes may actually improve a couple's chance of conceiving through IVF.

While there is increasing evidence demonstrating that lifestyle factors have deteriorating effects on fertility, little is known about the exact pathway mediating their effect on the success of ART [6–8]. Furthermore, few studies have specifically evaluated the combined effect of different lifestyle factors, including obesity, smoking or alcohol drinking, on early fertility treatment outcomes, such as quantity and quality of retrieved oocytes [9,10] which in turn is a robust surrogate marker for clinical success of IVF treatment [11,12]. Consequently, since negative lifestyle factors often co-occur, it cannot be ruled out that there is a synergistic effect, which in turn warrants further research. Hence, the primary aim of the present study was to elucidate the influence of individual, as well as cumulative effect of lifestyle factors on early reproductive outcomes after a first completed IVF cycle. A secondary aim was to also investigate the possible interaction of lifestyle factors with antral follicle count. By employing two different prospective cohorts, we were able to use one cohort to generate hypotheses (identify potential risk factors) and the other cohort to validate them.

Materials and methods

Design

This was a cohort study based on 2 different prospective cohorts.

Participants and recruitment

"Lifestyle study cohort" (hypothesis-generating cohort). The "Lifestyle study cohort" consists of women attending eight fertility clinics in Sweden (six public and two private).

Consecutive women ($n = 874$) were asked to participate in the study at their first visit to the clinic. The time of this first visit to the clinic was defined as 'baseline year'. Those who agreed to participate received a questionnaire (S1 and S2 Questionnaire) to complete either at the clinic or at home (returning it by mail in a prepaid envelope). The completed questionnaires were returned including the signed informed consent. Data collection started in May 2013 and ended in September 2015. Further details on this cohort can be found in a previous publication [13].

Of 874 eligible women, 747 (85.4%) agreed to participate and 466 (62.3%) completed the questionnaire. Follow-up data regarding the reproductive outcomes were obtained from the medical records of 442 women in January 2017. Only women who completed their first IVF treatment ($n = 242$) were included in the study. Women undergoing donor oocyte, donor sperm and preimplantation genetic diagnosis (PGD) cycles were excluded.

The patients underwent IVF according to standard stimulation protocols. Women in the study cohort underwent ovarian stimulation with the use of a long GnRH agonist protocol in 31.0% of treatments and a GnRH antagonist protocol in 69.0%, with recombinant FSH in 78.5% of treatments and hMG in 21.5%. Ovulation was triggered mostly with the use of subcutaneous hCG, while the use of GnRH agonist was limited only among cases at risk of ovarian hyperstimulation syndrome (OHSS). Embryologists have determined the total number of oocytes retrieved per cycle and evaluated the maturity of the oocytes. Only metaphase II oocytes (MII) were considered mature. Normal fertilization, indicated by the presence of two clearly distinct pronuclei and the embryo quality was assessed on the day of transfer. The cleavage stage embryos were scored based on cell number and the degree of fragmentation, according to the grading system of Istanbul Consensus Workshop on Embryo Assessment [14]. The Regional Ethical Review Board in Uppsala approved of the study (2012/278).

“UppStART study” cohort (validation cohort). The validation cohort was derived from the Uppsala-Stockholm Assisted Reproductive Technology (UppStART) study [15,16]; i.e. a prospective cohort study of couples undergoing their first IVF treatment in the greater Stockholm region (Stockholm and Uppsala County) ($n = 432$). Participants were recruited from one public and two private fertility and reproductive health clinics in Stockholm and one private clinic in Uppsala County, which serves a large volume of patients from Stockholm. Signed consent forms were sent to the UppStART research nurse at Karolinska Institute (KI) who monitored recruitment and questionnaire responses.

The participants were asked to answer an extensive web-based questionnaire within a few days of their clinical visit and prior to starting their IVF treatment, which included questions on sociodemographic, anthropometric and lifestyle factors. Of the total number of women who gave consent to participate in UppStART, 14% did not respond to the baseline questionnaire. Recruitment took place from September 2011 to December 2013 and participants were followed up until December 2014 ($n = 432$).

The IVF treatment protocols used in this study did not substantially differ from the one described above for the “Lifestyle study cohort” participants.

The Regional Ethical Review Board in Stockholm approved of the study (2011/230-31/1, 2011/1427-32, 2012/131-32, 2012/792-32, 2013/1700-32).

A flowchart describing participant progression through the study/IVF cycle is presented in Fig 1.

Study variables

Background, risk lifestyle and infertility-related variables. Age at start of cycle (years), education level [divided into low (non-university) and high (university level education)], and body mass index (BMI) (kg/m^2) (at the time of first visit to the fertility clinic) were assessed via

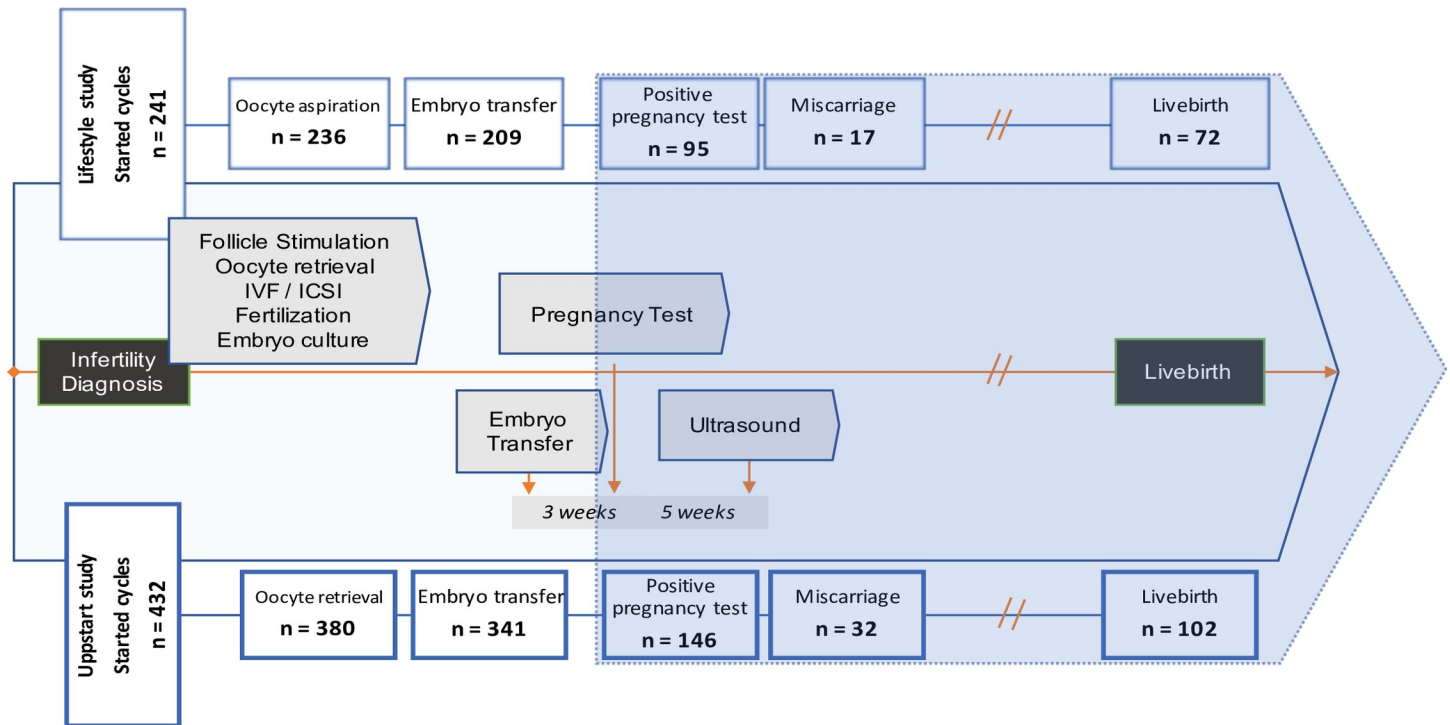


Fig 1. Flowchart of IVF cycles procedures included and their outcomes in the 2 study cohorts.

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medical records. Alcohol consumption was self-reported and defined as number of standard drinks per week. Questions in the “UppStART study cohort” were more detailed with regards to amount and type of alcohol. Smoking was defined as smoking currently or within the past year before their visit to the fertility clinic (yes/no). The daily intake of caffeine (mg) was estimated from the number of cups of coffee (77 mg/one cup of coffee or equivalent) in “UppStART study cohort”. In “Lifestyle study cohort” caffeine consumption in this study was assessed by asking women about their coffee consumption only. Physical activity level (PAL) was calculated using the validated ‘PAL’ method, with scores assigned according to different levels of reported occupational and leisure activity on the baseline questionnaire [17]. Cause of infertility was obtained from participants’ medical records at the start of the cycle and classified as male factor, female factor, and mixed/unknown causes. Duration of infertility was defined as the number of years trying to become pregnant. Antral follicle count (AFC) was defined as total antral follicles assessed by ultrasound examination. In this study, we used AMH and AFC as markers for ovarian reserve. Women in both study cohorts were tested for ovarian reserve using a combination of AFC and anti-Müllerian hormone (AMH) level at their first visit to the fertility clinic. Out of 242 women in the Lifestyle study 98.35% were tested for AFC and 82.24% were tested for AMH. Out of 432 women in the Upstart study 68.06% were tested for AFC and 79.87% were tested for AMH.

Predictor/exposure variables. We considered the following exposure variables as possible risk factors: age, BMI, smoking, alcohol consumption, daily caffeine consumption (only coffee) and physical activity score (PAL). In addition, the history of diagnosed depression (self-reported or via diagnosis registered in medical records) was also considered as one of the exposure variables.

Outcome variables. All outcome variables were derived from data originating from the participants’ medical records. The outcomes were total number of oocytes aspirated, number

of mature oocytes, number of embryos created, as well as number of utilizable embryos (i.e. transferred fresh and frozen).

Statistical analysis

All statistical analyses were performed using R 3.5.3. Data in the two cohorts were analyzed in an identical manner in order to determine if risk factors identified in the “Lifestyle study cohort” could be validated in the “UppStART study cohort” as having a cumulative additive effect. Baseline characteristics were presented as medians and interquartile range (IQR). To identify differences between the two cohorts, the non-parametric Mann Whitney test was used for continuous variables, while for the comparison of categorical variables, the Chi square test was conducted. For each of the outcomes and cohorts, a dataset consisting of the outcome and possible risk factors was created. Multiple imputations were used [18–20] in order to impute missing data and construct 20 imputed datasets for each outcome/cohort combination.

In the “Lifestyle study cohort”, a crude univariable quasi-poisson regression was performed evaluating the association between each of the outcomes in the study cohort and each of the risk factors. Additionally, identification of the effect modification of the risk factors by total AFC was attempted by using a likelihood ratio test. An adjusted multivariable analysis was then used, where all of the risk factors were included in a single model. Quasi-poisson regression was applied as the outcome variables were right-skewed. The effect estimates were pooled using standard statistical methodology for multiple imputation for nonresponse in surveys [21]. Variables that were significant at the $\alpha = 0,05$ level in the “Lifestyle study cohort” in either the crude or adjusted analyses were considered to be potential risk factors for the respective outcomes.

We then wanted to see if we (based on our findings from the “Lifestyle study cohort”) could create a numerical risk score that could be validated in the “UppStART study”. The following was performed for each outcome: if two or more potential risk factors were found in the “Lifestyle study cohort”, we then proceeded to run the following analyses in the “UppStART study”. We began by creating a new variable “number of risk factors.” If the potential risk factor was dichotomous and showed an incidence rate ratio (IRR) less than one in the “Lifestyle study cohort”, then the variable “number of risk factors” was increased by one if the person had the potential risk factor (i.e. if a variable is expected to reduce the number of oocytes/embryos, that variable is a risk factor). Likewise, if the potential risk factor was dichotomous and showed an incidence rate ratio (IRR) greater than one in the “Lifestyle study” cohort, then the variable “number of risk factors” was increased by one if the person did not have the variable (i.e. if a variable is expected to increase the number of oocytes/embryos, it is a risk factor to not have that variable). If the risk factor was continuous, then it was dichotomized at the median (i.e. “greater than the median”/ “lower than the median”). With regards to this model, we can interpret the coefficients as “percentage increase/decrease of outcome due to a one unit increase in the exposure”. For example, we can take a model with an outcome of “mature oocytes” and an exposure of one risk factor vs a baseline of 0 risk factors. If the $IRR = 1.04$ it means that women with one risk factor are expected (on average) to have 4% more mature oocytes per oocytes aspirated than women with zero risk factors. If the $IRR = 0.73$ it means that women with one risk factor are expected (on average) to have 27% less mature oocytes per oocytes aspirated than women with zero risk factors.

For each outcome, we then ran four quasi-poisson statistical regression models: Model 1: No exposures; model 2: Model 1 + number of risk factors as a categorical exposure; model 3: Model 2 + Total AFC and model 4: model 3 + interaction between BMI and Total AFC.

The significance of number of risk factors was tested by using a likelihood ratio test to compare Models 1 and 2. Additionally, identification of the effect modification of the number of risk factors by total AFC was attempted by using a likelihood ratio test comparing models 3 and 4.

Results

The participant's background characteristics, lifestyle, and infertility related characteristics are summarized in [Table 1](#). A total of 242 women who underwent their first fresh IVF cycle were evaluated in the "Lifestyle study cohort" and 432 women in the "UppStART study" validation cohort. The median age of the population at cycle start was lower in the "Lifestyle study cohort" [31.1 (IQR 6.8) years] compared to the validation cohort [34.0 (IQR 6.0) years]. There were more women with university education in the "UppStART study" validation cohort compared to the "Lifestyle study cohort". The most common cause of infertility was unexplained/mixed and this was similar in both study populations. Furthermore, median AMH was higher in the "Lifestyle study" cohort whereas median AFC was lower in that group compared to the "UppStART study" cohort. The median number of oocytes retrieved was 9 (IQR 6.5) in the "Lifestyle study cohort" and 8 (IQR 6.0) in the "UppStART study" validation cohort ([Table 1](#)).

Effect of individual lifestyle risk factors

Women with high BMI had an adjusted IRR for the number of aspirated oocytes corresponding to 0.98 (95% CI: 0.97, 1.00) which means that with each unit increase in BMI, the women had 2% fewer aspirated oocytes ([Table 2](#)). There was also a significant decrease in number of created and utilizable embryos with increasing BMI, [IRR 0.97 (95% CI: 0.95, 0.99) and 0.96 (95% CI: 0.94, 0.99) respectively ([S1 Table](#)). Women who smoked had 19% fewer aspirated oocytes; however, this result was not significant in the "UppStART study" validation cohort ([Table 2](#)). Interestingly, there was a decrease in the number of created embryos and number of utilizable embryos in relation to alcohol intake in "UppStART study" validation cohort ([S1 Table](#)).

Association between combined lifestyle risk factors and number of aspirated oocytes

Two potential risk factors were identified in the "Lifestyle study cohort": BMI and smoking ([Table 2](#)). In the "UppStART study" validation cohort, the variable "number of risk factors" (categorical) was found to be significant ($p = 0.007$). Women who had both risk factors had an IRR of 0.75 (95% CI: 0.61–0.94) for the number of aspirated oocytes (compared to a baseline of no risk factors), and consequently (on average) had 25% less aspirated oocytes than women without these risk factors ([Table 3](#)).

Association between lifestyle risk factors and number of mature oocytes

Two potential risk factors were identified in the "Lifestyle study cohort": BMI and smoking ([S1 Table](#)). In the "UppStART study" validation cohort, the combined variable "number of risk factors" was found to have a significant association ($p = .03$), and a woman who had both risk factors had an IRR of 0.78 (95% CI: 0.62–0.98), meaning that she would (on average) have 22% fewer mature oocytes than a woman with no risk factors ([Table 3](#)).

We also ran a sensitivity analysis to see the effect of the variables 'Total FSH dosage' and 'IVF agonist protocol' on the analysis. This information was only available for the Lifestyle study cohort. We therefore recreated [Table 2](#) for the outcomes of 'Number of aspirated oocytes' and 'Number of mature oocytes', including a third column where we included 'Total FSH dosage' and 'IVF agonist protocol' to the list of adjusted variables ([S2](#) and [S3](#) Tables). We found that BMI and smoking were unaffected by the inclusion of these two new confounders, highlighting the robustness of our findings.

Table 1. Background characteristics, lifestyle variables and variables relating to infertility and infertility treatment among the participants of the two cohorts.

| Characteristic | Lifestyle study N = 241 | | UppStART study N = 432 | | P-Value |
|---|----------------------------|-----------------|---------------------------|-----------------|---------|
| Age (years), median (IQR) | 31.1 | (27.9–34.7) | 34.0 | (31.0–37.0) | <0.001 |
| Level of education, n (%) | | | | | <0.001 |
| College/university | 153 | (64.3) | 334 | (78.8) | |
| Non-college/non-university | 85 | (35.7) | 90 | (21.2) | |
| Body Mass Index (BMI) in kg/m ² , median (IQR) | 23.7 | (21.4–27.9) | 22.7 | (20.9–25.6) | <0.001 |
| Any alcohol consumption, n (%) | 165 | (69.0) | 337 | (78.0) | 0.013 |
| Smoking ^a , n (%) | 78 | (32.4) | 148 | (35.3) | 0.493 |
| Smoking ^b , n (%) | 23 | (9.5) | 14 | (3.3) | 0.002 |
| Caffeine- daily ^c in mg/day, median (IQR) | 221.1 | (0.0–331.7) | 221.1 | (68.1–382.3) | <0.001 |
| Physical activity score ^d , median (IQR) | 1.7 | (1.7–1.8) | 1.7 | (1.6–1.8) | 0.064 |
| History of depression, n (%) | 42 | (17.6) | 58 | (13.4) | 0.183 |
| Cause of infertility, n (%) | | | | | 0.872 |
| Mixed/Unknown | 133 | (55.2) | 88 | (54.0) | |
| Male | 44 | (18.3) | 28 | (17.2) | |
| Female | 64 | (26.6) | 47 | (28.8) | |
| Duration of infertility (years), median (IQR) | 2.0 | (1.5–3.0) | 2.0 | (2.0–3.0) | 0.020 |
| Total AFC, median (IQR) | 10.0 | (7.0–14.0) | 16.0 | (10.0–24.0) | <0.001 |
| Total AFC < 7, N (%) | 50 | (21.0) | 24 | (8.2) | <0.001 |
| AMH, median (IQR) | 2.5 | (1.6–4.7) | 2.1 | (1.1–3.8) | 0.008 |
| FSH- total dose for stimulation, median (IQR) | 1513.5 | (1198.5–2250.0) | 1650 | (1206.2–2610.0) | 0.119 |
| Number of aspirated oocytes, median (IQR) | 9.0 | (6.0–12.5) | 8.0 | (5.0–11.0) | 0.003 |
| Number of mature oocytes, median (IQR) | 7.0 | (5.0–11.0) | 7.0 | (4.0–11.0) | 0.142 |
| Number of embryos created, median (IQR) | 4.0 | (2.0–6.5) | 4.0 | (2.0–7.0) | 0.621 |
| Number of embryos used, median (IQR) | 2.0 | (1.0–4.0) | 2.0 | (1.0–4.0) | 0.495 |

^a Smoking in general, currently or within the past year

^b currently smoking only.

^c 1 cup of coffee = 77mg caffeine

^d Physical activity score based on validated scale and reflecting different levels of occupational and leisure activity

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Interaction with AFC

While none of the cumulative risk factors had significant interactions with AFC, we did find that for the outcome of “number of mature oocytes per number of aspirated oocytes in IVF cycles” (in a model only containing BMI, AFC, and the interaction between BMI and AFC), BMI had a borderline-significant interaction with AFC in the Lifestyle study (p-value = 0.065, Table 4) and a significant interaction with AFC in the UppStART study (p-value = 0.038, Table 4). When using a model that adjusted for age, BMI, smoking, alcohol consumption, daily caffeine consumption, physical activity score and history of depression, the interaction between BMI and AFC had p-values of 0.086 and 0.045 for the Lifestyle study and UppStART study, respectively (other data not shown).

Discussion

Our study highlights the individual and combined impact of female lifestyle risk factors, mostly BMI and smoking, on the number of aspirated and mature oocytes in the first IVF

Table 2. Incidence rate ratio (IRR) and 95% Confidence intervals for number of aspirated oocytes and number of mature oocytes in relation to individual risk life-style factors in study cohorts.

| Outcome | Variable | Lifestyle study cohort | | | | UppStART study cohort | | | |
|-----------------------------|-------------------|-----------------------------|---------|--------------------------------|---------|-----------------------------|---------|--------------------------------|---------|
| | | Crude IRR (CI) ^a | p-value | Adjusted IRR (CI) ^b | p-value | Crude IRR (CI) ^a | p-value | Adjusted IRR (CI) ^b | p-value |
| Number of aspirated oocytes | BMI | 0.98 (0.97, 1.00)* | 0.019 | 0.98 (0.97, 1.00)* | 0.023 | 0.97 (0.95, 0.99)* | 0.008 | 0.97 (0.95, 0.99)* | 0.011 |
| | Smoking | 0.81 (0.70, 0.94)* | 0.005 | 0.79 (0.68, 0.93)* | 0.004 | 0.99 (0.85, 1.16) | 0.914 | 1.01 (0.86, 1.19) | 0.865 |
| | Age | 1.00 (0.99, 1.01) | 0.994 | 1.00 (0.98, 1.01) | 0.893 | 0.98 (0.96, 0.99)* | 0.010 | 0.98 (0.96, 1.00)* | 0.017 |
| | Alcohol | 1.01 (0.87, 1.17) | 0.873 | 1.04 (0.89, 1.20) | 0.651 | 0.87 (0.73, 1.05) | 0.142 | 0.90 (0.75, 1.08) | 0.260 |
| | Caffeine | 1.00 (1.00, 1.00) | 0.384 | 1.00 (1.00, 1.00) | 0.885 | 1.00 (1.00, 1.00) | 0.579 | 1.00 (1.00, 1.00) | 0.978 |
| | Physical activity | 0.63 (0.31, 1.30) | 0.212 | 0.62 (0.31, 1.26) | 0.187 | 0.87 (0.50, 1.51) | 0.617 | 0.75 (0.43, 1.31) | 0.319 |
| | Depression | 1.13 (0.95, 1.34) | 0.179 | 1.15 (0.97, 1.36) | 0.099 | 0.88 (0.71, 1.09) | 0.246 | 0.91 (0.73, 1.12) | 0.364 |
| Number of mature oocytes | BMI | 0.98 (0.96, 1.00)* | 0.019 | 0.98 (0.97, 1.00)* | 0.026 | 0.97 (0.95, 0.99)** | 0.018 | 0.97 (0.95, 0.99)* | 0.017 |
| | Smoking | 0.81 (0.69, 0.94)* | 0.006 | 0.79 (0.67, 0.93)* | 0.004 | 1.01 (0.85, 1.18) | 0.983 | 1.02 (0.86, 1.20) | 0.743 |
| | Age | 1.00 (0.98, 1.01) | 0.947 | 1.00 (0.98, 1.01) | 0.753 | 0.98 (0.96, 0.99)* | 0.010 | 0.98 (0.96, 1.00)* | 0.022 |
| | Alcohol | 1.05 (0.90, 1.22) | 0.529 | 1.08 (0.92, 1.26) | 0.335 | 0.82 (0.69, 0.99)* | 0.035 | 0.84 (0.70, 1.01) | 0.067 |
| | Caffeine | 1.00 (1.00, 1.00) | 0.378 | 1.00 (1.00, 1.00) | 0.986 | 1.00 (1.00, 1.00) | 0.889 | 1.00 (1.00, 1.00) | 0.472 |
| | Physical activity | 0.71 (0.34, 1.49) | 0.365 | 0.75 (0.34, 1.47) | 0.351 | 0.81 (0.46, 1.44) | 0.474 | 0.70 (0.40, 1.22) | 0.207 |
| | Depression | 1.13 (0.94, 1.34) | 0.192 | 1.16 (0.97, 1.38) | 0.105 | 0.86 (0.69, 1.08) | 0.196 | 0.89 (0.71, 1.11) | 0.300 |

^a Incidence rate ratio + 95% CI for the crude univariable analysis

^b Incidence rate ratio + 95% CI for the adjusted analysis (adjusted for age, BMI, smoking, alcohol consumption, daily caffeine consumption, physical activity score and history of depression)

* $p < 0.05$

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treatment cycle. To our knowledge, this is the first study to also demonstrate a possible interaction of BMI in relation to AFC measurement when exploring its effect on the number of mature oocytes per number of aspirated oocytes in IVF cycles.

Healthy and unhealthy lifestyle behaviors tend to occur in clusters [22]. A healthy way of life can be characterized by an accumulation of multiple healthy lifestyle choices and vice versa. Therefore, researchers have lately adopted a more holistic approach to lifestyle and seek

Table 3. Incidence rate ratio (IRR) and 95% Confidence intervals (CI) for IVF treatment outcome in association with cumulative risk lifestyles.

| Outcome | Risk factors included ^a | Exposure category | Crude IRR (95% CI) ^b | p-value for comparison against reference ^c | p-value for entire categorical variable ^d | p-value for interaction with AFC ^e |
|-----------------------------|------------------------------------|-------------------|---------------------------------|---|--|---|
| Number of aspirated oocytes | BMI, Smoking | 0 risk factors | Ref. | | 0.007 | 0.568 |
| | | 1 risk factor | 0.86 (0.73–1.00) | 0.057 | | |
| | | 2 risk factors | 0.75 (0.61–0.94)* | 0.013 | | |
| Number of mature oocytes | BMI, Smoking | 0 risk factors | Ref. | | 0.031 | 0.431 |
| | | 1 risk factor | 0.88 (0.74–1.04) | 0.124 | | |
| | | 2 risk factors | 0.78 (0.62–0.98)* | 0.034 | | |

^a Risk factors were identified in the “Lifestyle study” cohort

^b Validation models were run in the “UppStART cohort”

^c Single wald p-value for the pairwise comparison of having X risk factors against the reference of 0 risk factors

^d Likelihood ratio test p-value for the categorical variable “number of risk factors”

^e Likelihood ratio test p-value for the interaction of continuous AFC with the categorical variable “number of risk factors”

* $p < 0.05$

<https://doi.org/10.1371/journal.pone.0221015.t003>

Table 4. Interaction between individual risk lifestyle factors with Antral Follicle Count (AFC) in affecting number of mature oocytes (IRRs with related 95% confidence intervals).

| Outcome | Variable | Lifestyle study cohort | | | | | UppStART study cohort | | | | |
|--------------------------|-------------------|------------------------|----------------------------|---------------------------|-------------------------------|---------------------------|------------------------|----------------------------|---------------------------|-------------------------------|---------------------------|
| | | Crude IRR ^a | Crude P-value ^b | Adjusted IRR ^c | Adjusted P-value ^d | Crude interaction P-value | Crude IRR ^a | Crude P-value ^b | Adjusted IRR ^c | Adjusted P-value ^d | Crude interaction P-value |
| Number of mature oocytes | BMI | 1.00 (0.99, 1.01) | 0.948 | 1.00 (0.99, 1.01) | 0.923 | 0.065 | 1.00 (0.99, 1.01) | 0.780 | 1.00 (0.99, 1.01) | 0.964 | 0.038 |
| | Smoking | 0.99 (0.94, 1.04) | 0.627 | 0.98 (0.93, 1.04) | 0.583 | 0.034 | 1.01 (0.97, 1.06) | 0.559 | 1.02 (0.97, 1.06) | 0.498 | 0.743 |
| | Age | 1.00 (1.00, 1.01) | 0.646 | 1.00 (1.00, 1.01) | 0.850 | 0.160 | 1.00 (0.99, 1.00) | 0.824 | 1.00 (1.00, 1.01) | 0.883 | 0.766 |
| | Alcohol | 1.05 (1.00, 1.11) | 0.070 | 1.06 (1.00, 1.12) | 0.058 | 0.303 | 0.95 (0.91, 1.00)* | 0.049 | 0.94 (0.90, 0.99)* | 0.026 | 0.380 |
| | Caffeine | 1.00 (1.00, 1.00) | 0.960 | 1.00 (1.00, 1.00) | 0.701 | 0.559 | 1.00 (1.00, 1.00) | 0.209 | 1.00 (1.00, 1.00) | 0.157 | 0.664 |
| | Physical activity | 1.07 (0.83, 1.38) | 0.617 | 1.08 (0.83, 1.40) | 0.556 | 0.051 | 0.93 (0.81, 1.08) | 0.363 | 0.92 (0.80, 1.07) | 0.271 | 0.908 |
| | Depression | 0.99 (0.93, 1.05) | 0.791 | 1.00 (0.94, 1.06) | 0.954 | 0.850 | 0.99 (0.93, 1.05) | 0.700 | 0.99 (0.93, 1.05) | 0.700 | 0.844 |

^a Incidence rate ratio + 95% CI for the crude univariable analysis

^b Likelihood ratio test p-value for the crude univariable analysis

^c Incidence rate ratio + 95% CI for the adjusted analysis

(adjusted for age, BMI, smoking, alcohol consumption, daily caffeine consumption, physical activity score, and history of depression)

^d Likelihood ratio test p-value for the adjusted analysis

* p<0.05

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to evaluate the cumulative effect of lifestyle behaviors on health outcomes [23]. Couples who are trying to conceive are usually advised to make lifestyle modifications, but this advice may be difficult or even impossible for some women to follow [7], leading often to thoughts of self-blame as a coping mechanism [24]. It is therefore important to assess the impact of lifestyle factors and their potential interaction with ovarian reserve, with the goal to give individually tailored clinical advice. Although there are several studies addressing the impact of lifestyle risk behaviors on various IVF treatment outcomes, such as pregnancy rate or livebirth rate [3,25,26] only a few of them evaluate the association between lifestyle behaviors and number and/or quality of aspirated oocytes in the IVF setting [27].

Based on the results of the current study the effects of smoking and BMI on IVF early treatment outcomes appear to be cumulative. A higher number of negative risk lifestyle factors is correlated to a significant decrease in number of aspirated and mature oocytes. It is also interesting to note the interaction of BMI with AFC, suggesting that at appropriate levels of ovarian reserve, the impact of BMI is possibly not so important. These results could contribute to individually tailored information to couples seeking treatment.

The existing literature is inconsistent as to whether obesity affects reproduction through mechanisms involving mostly the ovaries or maybe even the uterus [27–30]. The impact regarding oocytes seems to be more pronounced; there is an increasing body of evidence

demonstrating a negative correlation between number of retrieved, as well as mature (MII) oocytes in obese women implying a harmful effect of the metabolic environment on retrieved oocyte quantity and quality [27,31–34]. However, in a register-based cohort study, the effect was apparent only in first treatment-cycles, whereas no effect was observed on the following treatment-cycles (\geq two) [35].

Nicotine on the other side may act as a mutagen and possible oocyte toxin. Fuentes et al., reported a proportional decrease of the number of oocytes when follicular fluid cotinine levels (i.e. a metabolite of nicotine which remains present in the body for prolonged periods) increased [36]. Furthermore, increased levels of reactive oxygen species (ROS) in follicular fluid, probably promoted by smoking exposure over a long period of time, correlate with immature oocytes and lower quality embryos, a finding that further strengthens our findings [37]. Firns et al demonstrated that basal FSH increased in tandem with years of cigarette smoking resulting, in turn, to lowered retrieved oocyte numbers and fertilization rates [10]. Our findings are in corroboration with these results.

In this study we used AMH and AFC as markers for ovarian reserve. There is still a lack of consensus regarding the established range for the low, and especially normal and above normal ovarian reserve (i.e. based solely on ovarian reserve markers without considering the total oocytes retrieved, “Bologna criteria” etc) [38]. However, there is mounting evidence to support the use of AMH (0.5 to 1.1 ng/ml) and fair evidence to support that a low antral follicle count (5–7) has moderate to high specificity as a screening test for poor ovarian response [39].

The effect of age on the number of aspirated oocytes in our study was statistically significant in the “UppStART study” validation cohort (S1 Table). This cohort includes a wider age range, (IQR; 31.0 to 37.0) and a higher age median (34.0 years) of women compared to the “Lifestyle study cohort”. In the Lifestyle cohort, which mainly included public fertility clinics, women were younger and with a narrow age range, rendering the effect of age less apparent. Despite what one would expect, median AMH was higher in the “Lifestyle study” cohort whereas median AFC was lower in that group compared to the “UppStart study” cohort (Table 1). We believe that this discrepancy could probably be due to either the fact that the “UppStart study” cohort actually had a lower BMI despite their higher age, the small sample size of the “Lifestyle study” cohort, or due to missing data regarding reported AFC in the “UppStART study” cohort. These two markers of oocyte reserve usually correlate, but they are affected by both age (favorable in the “Lifestyle study”) and somewhat by BMI (favorable in the “UppStart study”), probably differentially impacting AMH and AFC and accounting for the observed differences between the cohorts. In order to confirm the hypothesis that AMH in relation to age was similar between the two cohorts, we ran a linear regression with AMH as the outcome, a binary variable demarcating cohort ownership as the exposure, and continuous age as a covariate. We found that the cohort variable had a p-value of 0.22, which suggested that there was no evidence that AMH differed between cohorts when age was accounted for. Taking that into account, we chose to report even other variables that indirectly mirror the ovarian reserve, such as total FSH dosage used per treatment which did not differ between the two cohorts. We believe thus, that these two cohorts are clinically comparable concerning ovarian reserve. We also believe more in the clinical importance of ovarian reserve differences in different ages which have been even documented in previous research [39]. Age is of course already recognized to be the most prominent predictor for success after IVF. Female fertility peaks at the age of 22 and subsequently decreases until around the age of 38, where after it falls more rapidly until menopause [40].

Regarding alcohol consumption, a decrease in number of created embryos and number of embryos available for transfer (fresh or frozen-thawed), were detected in the “UppStART study” validation cohort (S1 Table). Surprisingly, this decrease could not be detected in the

“Lifestyle study cohort”, perhaps because of socioeconomic and age differences in the two cohorts, as well as different phrasing of the specific questions in the questionnaire in the “UppStART study” validation cohort, which were more detailed with regards to amount and type of alcohol. Similar results were shown in a smaller study from Southern California which showed a decrease in the number of aspirated oocytes and number of fertilized oocytes among alcohol drinkers [41].

Furthermore, in our study, there were no statistically significant associations between caffeine or physical activity and IVF early treatment outcomes. However, caffeine consumption in this study was assessed by asking women about their coffee consumption only and not about other caffeine-containing beverages. Caffeine consumption has been shown to be negatively associated with the number of oocytes collected [42] but Abadia et al found no further influence of caffeine on intermediate or clinical endpoints of ART [43]. In addition, we did not ask about specific types of physical activity, but rather ascertained the number of hours of activity per week for all types of vigorous or moderate physical activity combined. More specifically, increasing amounts of exercise appear to have a variable effect on IVF outcomes [44], but certain categories of exercise may adversely affect IVF-related pregnancy rates [26,45].

Limitations and strengths

Limitations include the self-reported data collected through study participant questionnaires for some of the lifestyle variables. It is well known for example that participants tend to under-report non-socially desirable behaviors, such as alcohol intake and smoking, possibly resulting in misclassification of some individuals. However, that is not the case for the details of IVF treatment, where all data were retrieved from participants’ medical records retrospectively and are thus highly reliable. This study did not include the corresponding male variables on lifestyle, which is a limitation since fertilization rates are affected by semen quality, which is in turn strongly associated to lifestyle behaviors in the partner. Furthermore, the relatively small sample size, especially in the “Lifestyle study cohort”, might introduce some problems with statistical power. This might be reflected in the borderline statistical significance of some of the results, where, on the other hand, notable and stable trends are demonstrated. Despite these shortcomings, this study is one of the largest on the field evaluating lifestyle factors, their interaction and possible impact on assisted reproductive early treatment outcomes, and uses a larger, validation cohort from the same geographic area to confirm the study findings. Participation rate was higher than in many other studies in the field [46].

Among the strengths of the study is the prospective design with complete follow up and the simultaneous inclusion of several lifestyle risk variables. Furthermore, by only exploring the first fresh IVF treatment cycle of every patient who met the inclusion criteria, the negative effects resulting from the history of repeatedly failed or cryopreserved cycles were minimized. In addition, we used one cohort for hypothesis generation, and another to validate our findings, adding strength to the validity of our findings. The sample of women with fertility problems was drawn from both public and private fertility clinics, which makes the sample representative regarding women undergoing fertility treatment in mid-Sweden and minimizes the risk for selection bias.

Conclusions

The findings of this study point both to an individual as well as a cumulative effect of smoking and BMI on the number of aspirated oocytes and the number of mature oocytes in fresh IVF treatment-cycles. AFC might modify associations between BMI and the proportion of mature oocytes in relation to the total number of aspirated oocytes. These negative lifestyle factors are

easy to detect at an early stage of the assessment process and might allow for optimization of the early treatment outcome. Whether women may be able to improve the outcome of fertility treatment by quitting smoking and losing weight must be further investigated.

Supporting information

S1 Table. Incidence rate ratio (IRR) and 95% Confidence intervals (CI) for IVF treatment outcomes in relation to risk lifestyle factors with and without denominator.

(DOCX)

S2 Table. Sensitivity analysis for the outcome number of aspirated oocytes including even FSH total dose and IVF protocol in the model.

(DOCX)

S3 Table. Sensitivity analysis for the outcome number of mature oocytes including FSH total dose and IVF agonist protocol in the model.

(DOCX)

S1 Questionnaire. Lifestyle study- Swedish.

(PDF)

S2 Questionnaire. Lifestyle study- English.

(PDF)

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