AUCTORES
Globalize your Research

Journal of Obstetrics Gynecology and Reproductive Sciences
John L Yovich

Open Access

Research Article

The Effect of Caffeine Consumption on Fertilization and IVF Outcomes: A Review and Presentation of Original Data

Syeda Zaidi[1], Karen A Joesbury[1,2], Andy Lee[2], Peter M Hinchliffe[1] and John L Yovich[1,3]*

[1]PIVET Medical Centre, Perth, Western Australia 6007

[2]Department of Epidemiology and Biostatistics, School of Public Health, Curtin University, Perth Western Australia 6845

[3]School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin University, Perth, Western Australia 6845

***Corresponding Author;** John L Yovich, School of Pharmacy and Biomedical Sciences, Faculty of Health Sciences, Curtin University, Perth, Western Australia 6845. **E-mail;** jlyovich@pivet.com.au

Received date: August 23, 2019; **Accepted date:** September 17, 2019; **Published date:** September 20, 2019

Citation: Zaidi, S., Joesbury, K.A., Lee, A., Hinchliffe, P.M., Yovich, J.L. (2019) The Effect of Caffeine Consumption on Fertilization and IVF Outcomes: a Review and Presentation of Original Data. J. Obstetrics Gynecology and Reproductive Sciences, 2(3); DOI:10.31579/2578-8965/022

Abstract

Caffeine is widely consumed by both men and women but its pharmacological effects have not been well studied in the area of assisted reproduction. Various authorities indicate that caffeine consumption up to 400 mg per day is safe, but caution is advised for women who are, or are contemplating pregnancy and they should keep their intake under 200 mg per day. Our lifestyle studies at Curtin University showed adverse effects from caffeine consumption in both men and women. For men, fertilization rates were negatively associated with caffeine consumption ($P<0.05$) as well as IVF-related stress ($P<0.005$). Furthermore, caffeine negated the beneficial effect of male alcohol consumption, comprising mainly beer, on fertilization. For women, fertilization rates were negatively associated with caffeine consumption ($P<0.005$) and smoking history in years ($P<0.001$). However, our studies could not show any effect of caffeine consumption on the chance of pregnancy or miscarriage up to week 12 from either male intake (up to 4495 mg per week) or female intake (up to 2706 mg per week). We conclude that there is likely to be an effect from caffeine on one-Carbon metabolism and future studies need to interrogate the concomitant nutritional intake of B-vitamins and serum homocysteine levels, which can indirectly indicate deficiency or interference with this important metabolic pathway.

Keywords: caffeine; IVF; fertilization; pregnancy; B-vitamins; one-carbon metabolism; homocysteine

Running Title: Caffeine reduced fertilization, probably by interfering with 1-Carbon metabolism.

Introduction

Caffeine is probably the most frequently ingested pharmacologically active substance in the world, found in common beverages (coffee, tea, soft drinks), products containing cocoa or chocolate, and medications [1]. Maximum caffeine concentrations in blood are reached within 1–1.5 hours following ingestion and readily distributed throughout the entire body. It crosses the blood–brain barrier, through the placenta into amniotic fluid and the fetus, and into breast milk. Caffeine has also been detected in semen [2,3] as well as follicular fluid [4].Caffeine is metabolised by the liver via the hepatic enzyme cytochrome P450 1A2 (CYP1A2) which de-methylates caffeine to produce the primary metabolite paraxanthine (84%), followed by theobromine (12%) and theophylline (4%) [5]. Caffeine's half-life is approximately 4 to 6 hours but this can vary depending on various factors [6]. For example, smoking increases caffeine metabolism by increasing both CYP1A2 activity and drug elimination, alternatively, pregnancy slows caffeine metabolism and increases its half-life [7,8].

The primary molecular action of caffeine is via nonspecific binding to adenosine G protein-coupled receptors due to caffeine being chemically similar in structure to adenosine [9,10]. The effects associated with caffeine-mediated inhibition of normal adenosine signalling include increased release of norepinephrine, serotonin, dopamine and catecholamines [6].It has been reported that moderate caffeine intake at a dose of 400 mg/day is not associated with adverse effects such as general toxicity, cardiovascular effects, changes in adult behaviour, increased incidence of cancer nor effects on male fertility [1]. The same article also proposed that women within the reproductive age can be defined as an 'at risk' group. As such they may require specific advice on moderating their caffeine intake and recommended that caffeine intake for women who plan to become, or are currently, pregnant should not exceed 300 mg/day. However, to date, there has been no definitive answer provided regarding the effects of caffeine consumption in relation to assisted reproduction technologies (ART), in particular that concerning in vitro fertilization (IVF) and embryo transfer (ET). This review aims to describe an original study undertaken as part of a thesis for Doctorate of Philosophy (PhD) at Curtin University [11] as well as reviewing further published findings examining the effect of caffeine consumption on ART outcomes. The Curtin Study examined a range of factors potentially impacting on ART outcomes with several of these already published [12,13]. This report will focus specifically on the findings regarding caffeine consumption and other potential lifestyle confounders.

Methods/Study design

By design, the Curtin study was a prospective cohort. Ethical approval to conduct the proposed study was obtained from the

Western Australian Reproductive Technology Council (WARTC) as well as from the Curtin University Human Research Ethics Committee (CUHREC) and from the Cambridge Private Hospital Ethics Committee (CPHEC) which acted as PIVET Medical Centre's Human Reproductive Ethics committee at the time. From January 1997 to August 1998, 351 couples who commenced their first IVF treatment at PIVET Medical Centre were recruited into this study. They comprised 281 females and 247 males.

The couples undergoing IVF treatment were recruited and followed through to pregnancy outcome, including miscarriage and completion of the first trimester with first trimester screening (FTS) to determine a viable pregnancy and risk for fetal malformations. Early in the course of IVF treatment, measures of lifestyle were ascertained from both parents. For the purpose of this review, the focus will be on the couple's reported caffeine consumption. As couples proceeded through the treatment stages of IVF, the intermediate clinical outcomes of oocyte production, fertilization rate and ET outcomes were documented. Sixteen days post-ET, pregnancy status was determined by hormonal analysis. By definition, pregnancy was defined as a beta-HCG level $\geq$ 25 IU/L at 16 days post embryo transfer. Women diagnosed as pregnant at this stage were monitored until week 12 of pregnancy. Multivariate statistical methods were used to determine the impact of lifestyle factors on these clinical outcomes of IVF treatment.

Data collection on lifestyle variables was self-reported but was fastidiously obtained through the Lifestyle Questionnaire/Diary (LQ/D). The LQ/D comprised of two parts: 1) questionnaire and 2) diary. The first part was a four-page questionnaire that was used to obtain data on demographic information not available from patient records, including birth place and education. It was also used to acquire data on smoking history, including years of tobacco consumption, cigarette brand, as well as information on usual tea and coffee consumption. Patients were instructed to complete the questionnaire on Day 4 of the female patient's treatment cycle. The second part of the LQ/D was a 7-day, questionnaire-style diary. Patients were requested to commence the diary on Day 4, after completing the questionnaire, and continue through to Day 10. The self-reported diary entries were validated by cross-checking from the Curtin researchers at the time of submission.

Tobacco consumption, caffeine intake and stress levels were included in the data collected in the diary each day. Two general appraisal questions were devised to separate the stress experienced due to 1) daily living in general and 2) IVF treatment. Each required the respondent to indicate their stress level on a scale from 0 ('absolutely no stress') to 10 ('most stress possible'). For each criteria of stress, total weekly levels were calculated by simply summing the seven daily scores, creating a possible value range from 0 to 70.

Data on caffeine consumption included intake from coffee, tea, cola and iced coffee beverages. In accord with the recommendations of Schreiber et al. (1988) (14), the diary was devised so as caffeine intake took account of 1) decaffeinated beverages, 2) brewing methods of coffee, 3) the proportion of the beverage consumed, and 4) caffeine-containing prescription and 'over-the-counter' medication.

To quantify the amount of caffeine consumed, the Caffeine Survey (1995) [15] was used to convert the self-reported lifestyle data from the LQ/D into total weekly caffeine consumption in mg. The Caffeine Survey (1995) was based on a submission by the Health Department of Western Australia (WA) between June and December 1994 of 107 food samples to the Chemistry Centre of WA for caffeine analysis. The caffeine content of 107 food samples including beverages were listed in the survey.

In order to use the caffeine values of the instant coffee beverages listed in the Caffeine Survey (1995), the Curtin researchers had to convert the various teaspoon measures into grams of instant coffee. A project was undertaken and conducted at Curtin University of Technology, WA, in the School of Public Health food science laboratory. The researchers concluded that the mean weight of instant coffee in a level and heaped teaspoon was 1.26g and 2.10g, respectively **(Supplemental Table S1).**

Instant coffee brand	**weight (gms)**					
	first	second	third	fourth	fifth	mean
Level teaspoon						
International Roast	1.23	1.27	1	1.19	1.22	1.18
Nescafe 43 Blend	1.29	1.47	1.5	1.31	1.24	1.36
Moccona Indulgence	1.31	1.19	1.26	1.2	1.3	1.25
				grand mean		**1.26**
Heaped teaspoon						
International Roast	2.49	2.23	2.26	2.2	2.06	2.25
Nescafe 43 Blend	2	2.07	2.09	2.11	1.92	2.04
Moccona Indulgence	1.94	2.08	2.01	2.05	1.97	2.01
				grand mean		**2.1**

Estimated Average Grams of Instant Coffee in a Teaspoon

The Caffeine Survey (1995) listed the caffeine content of ten brands of caffeinated instant coffee beverages, each being made from a serve size of 2g (Supplemental Table S2). The mean caffeine content of these ten brands was 72mg per 2g serve. The average caffeine content of four brands of decaffeinated instant coffee was 3mg per 2g serve **(Supplemental Table S2).**

	Brand of instant coffee	**product label %m/m(a,b)**	**caffeine (mg) per 2gm serve (c)**
1	Bushells	3.8	76
2	Bushells Pablo	4	80
3	Home Brand	3.8	76
4	International Roast	4	80
5	Maxwell House Special Cup	3.1	62
6	Moccona freeze dried	3.2	64
7	Nescafe Blend 43	3.5	70
8	Nescafe Gold Blend freeze dried	3.1	62
9	Nescafe Espresso	3.6	72
10	Savings	3.8	76
		mean	71.8
	Brand of decaffeinated instant coffee	**product label %m/m**	**caffeine (mg) per 2gm serve**
1	Cafe Hag instant coffee	2400 mg/kg	5
2	International Roast instant coffee	0.09% m/m	2
3	Moccona instant coffee granules	1600 mg/kg	3
4	Nescafe Decaf	0.14% m/m	3
		mean	3

Supplemental Table S2: Caffeine Content of Caffeinated and Decaffeinated Instant Coffee

For tea beverages, the survey applied a caffeine value of 52mg per 2g teabag (Supplemental Table S3), which was the mean calculated from the nine brands of tea bag investigated.

	Tea bag sample	product label %m/m (a,b)	caffeine (mg) per 2m teabag (c)
	Caffeinated		
1	Brooke Bond PG tips two cup size tea bag	3.1	62
2	Bushells blue label tea cup bag	2.8	56
3	Bushells extra strong round tea bags	2.8	56
4	Dilmah 100% tea cup bags	1.5	30
5	Earl Grey - Twinings tea bags	3.0	60
6	English Breakfast - Twinings tea bags	3.4	68
7	Nerada tea bags	2.0	40
8	Tetley tea chest tea bags	2.4	48
9	Tetley tea cup bags	2.5	50
		Mean	52
	Decaffeinated		
1	Lipton naturally decaf teabag (<4.0% caffeine)	0.38	8
2	Tetley decaffeinated tea bag (<0.3% caffeine)	2.1	42
3	Tetley decaffeinated tea bag (<0.3% caffeine)	0.05	1
		Mean	17

Supplemental Table S3: Estimated Average Caffeine Content of Tea Bags

a stated on product label, as reported in the 1995 Caffeine Survey
b 1% m/m is equal to 10,000mg/kg
c value reported in the 1995 Caffeine Survey

A mean caffeine value of 51mg was given for tea beverages made from loose tea leaves based on 4 loose tea brands. For decaffeinated tea bags, a mean caffeine value of 17mg was given (Supplement Table S3). Kola drinks were also included as part of the Caffeine Survey (1995). Based on three Kola drinks, a mean caffeine value of 10.2 mg/100ml was given **(Supplemental Table S4)**

	Kola Drinks	Caffeine mg/L (a)	Serving size (mL)	Caffeine (mg) per serve	Caffeine (mg) per 100mL
1	Coca Cola	96	375	36	9.6
2	Dr Pepper	110	354	39	11.0
3	Pepsi Cola	100	375	38	10.1
				mean	10.2
	Diet				
1	Diet Coca Cola	140	375	53	14.1
2	Diet Pepsi	110	375	41	10.9
				mean	12.5
	Caffeine free				
1	Diet Pepsi	<1	375	<1	0
2	Diet Coke	<1	375	<1	0
				mean	0

Supplemental Table S4: Caffeine Content of Kola Drinks

(a) as reported on the product label

For the main outcome measures of the study, multivariate methods of data analyses were used to control for patient and treatment variables in the examination of the effect of lifestyle factors on the following clinical outcomes: 1) number of oocytes retrieved by transvaginal oocyte aspiration (oocytepick-up [OPU]), 2) fertilization, measured as the number of oocytes fertilized against the number of oocytes inseminated, 3) beta-human chorionic gonadotrophin (HCG) pregnancy, 16 days post-embryo transfer, and 4) <12week pregnancy loss following confirmation of beta-HCG pregnancy. As a measure of ovarian reserve, serum basal follicle stimulating hormone (FSH) levels were also investigated as a dependent variable. Lifestyle factors included years of cigarette smoking (smoke years), tobacco, alcohol, caffeine and fruit and vegetable consumption, and stress from daily living and IVF treatment. Scatterplots and Pearson's correlation coefficients (r) were used to investigate linear associations between continuous independent variables and oocyte production. To generate a multiple logistic regression model, the best subset of twelve variables (including the constant) was generated using various model fitting strategies: full model (forcing the fit of all of the variables into the model), backward elimination (with sequential removal of variables from the full model based on the highest P value), forward selection, stepwise selection (with sequential variable selection based on the lowest P value in the full model) and exhaustive search. All models contained female nicotine and smoke years and also male nicotine, alcohol, fruit and vegetable and finally male IVF stress.

Results

Figure 1 shows the flow chart for the 351 couples recruited into the study, whereby 61 couples achieved a pregnancy with a normal FTS evaluation and were ongoing at 12 weeks. Information was obtained on the weekly levels of caffeine consumption from 238 of the 281 women initially recruited. Overall a total of 227 of the 238 women consumed caffeine during the week of the diary, ranging from 4 to 3186 mg/week **(Figure 2).** The median caffeine consumption was 800 mg/week.

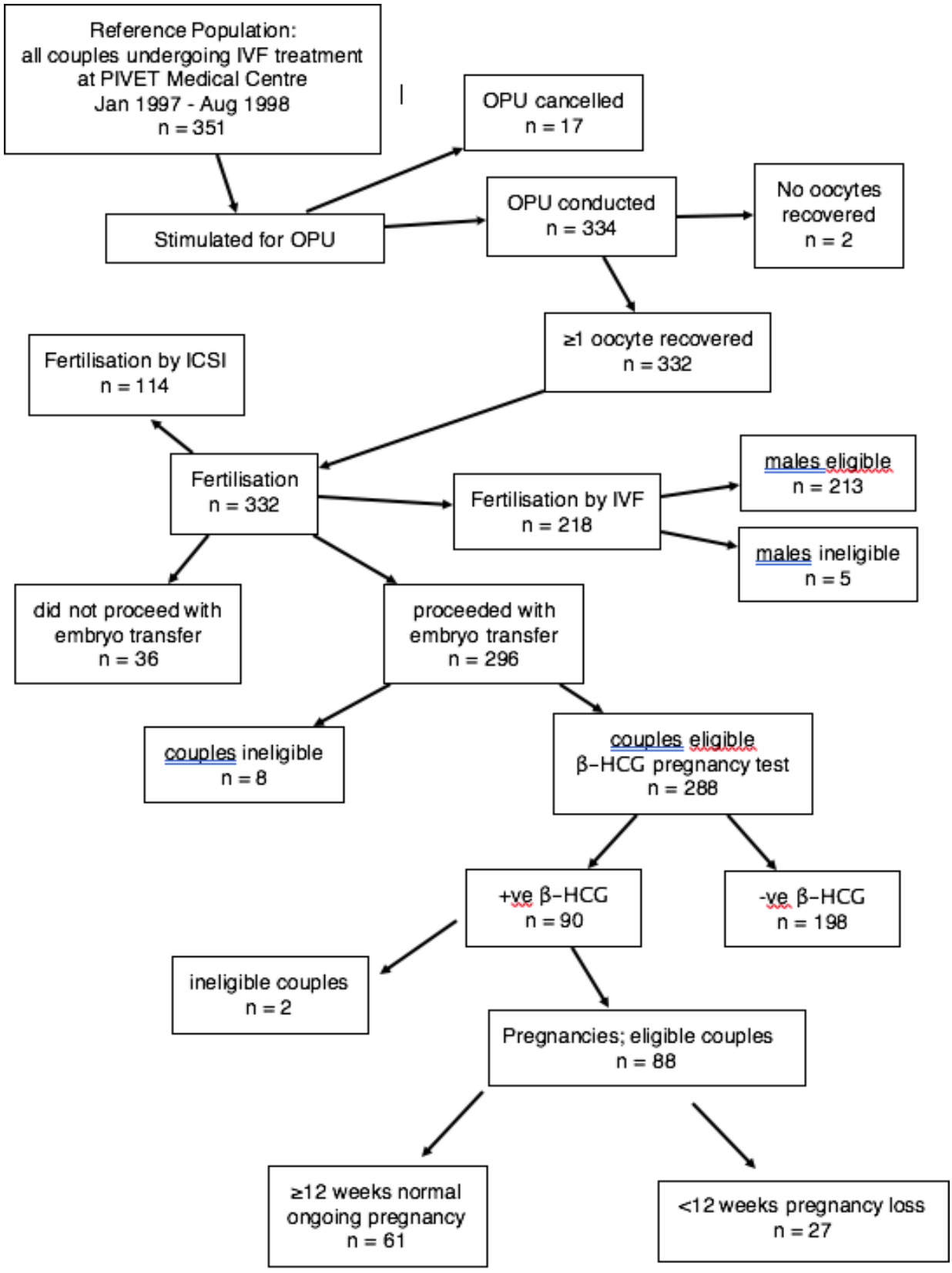

Figure 1: Flow chart of reference population (n=351 couples), study samples and subsamples. OPU; oocyte pick-up. Pregnancy diagnosed at β-HCG level ≥25 IU/L

Negative linear associations were suggested, albeit weak between number of oocytes and caffeine consumption (r=-0.15, P=0.02) and alcohol consumption (r=-0.15, P=0.02) **(Figure. 2).**

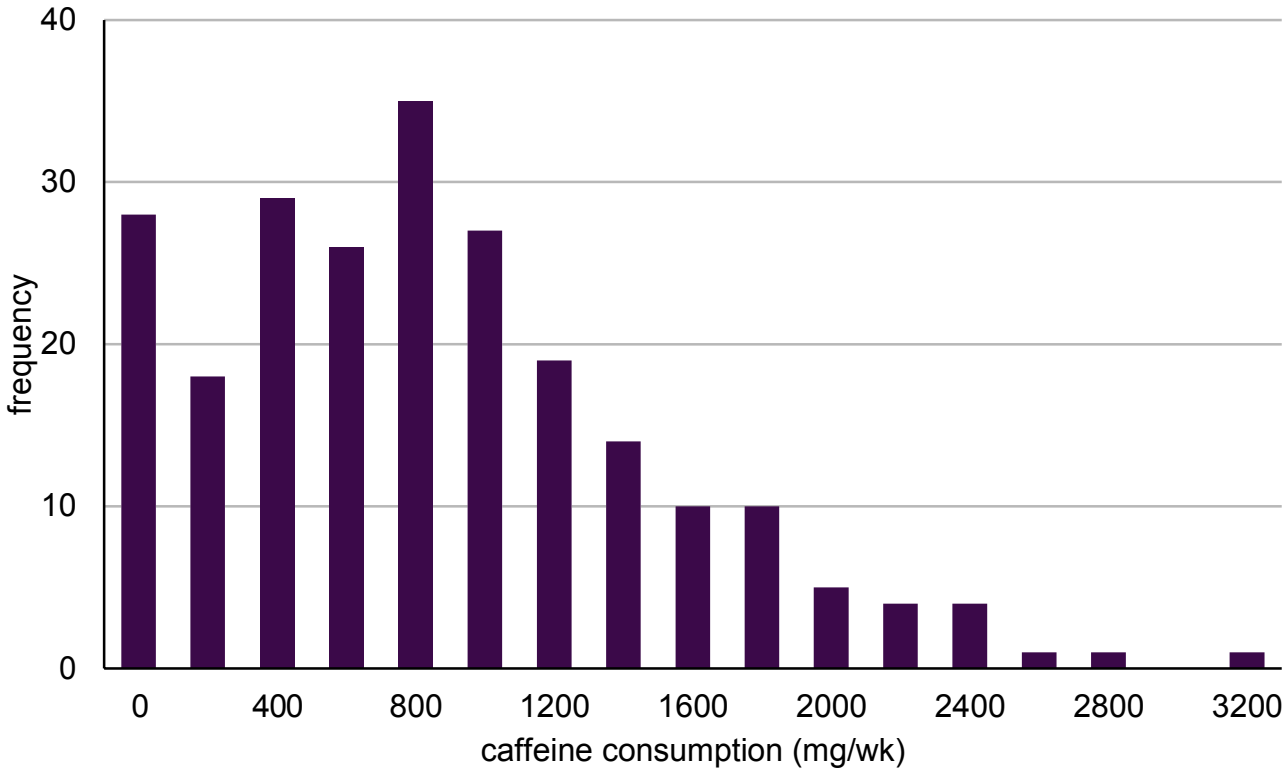

Figure 2: Histogram of Caffeine Consumption

With regards to fertilization, 6 women and 7 men reportedly did not consume caffeine. Of the women who did consume caffeine, their intake ranged between 4 and 2706 mg/week, with a mean value of 902 mg/week (Table 1). For male caffeine consumers, their intake ranged between 20 to 4495 mg/week, with a mean of 1152 mg/week (Table 1).

Gender	Variable	Min	Max	Median	IQR	Mean	SD
Female	Smoke Years	0	25	1	10	5.2	-
	Caffeine (mg/wk)	0	2706	846	-	902	644
	Caffeine (mg/wk)[#]	4	2706	846	-	939	630
	Alcohol (std drinks/wk)	0	27	0.8	5	3	-
	Daily Stress	0	57	19	-	19.4	11.4
	IVF Stress	0	45	17	-	17.7	11.9
Male	Smoke Years	0	29	0	12	6.2	-
	Caffeine (mg/wk)	0	4495	1033	-	1152	845
	Caffeine (mg/wk)[#]	20	4495	1033	-	1207	825
	Alcohol (std drinks/wk)	0	79	7	11.8	9.9	-
	Daily Stress	0	53	19.8	-	21.4	12.6
	IVF Stress	0	63	7	16	11.5	-

Table 1: Descriptive Statistics of Lifestyle Variables

[#] excluding those who did not consume caffeine (n=6 women and n=7 men)

Univariate analysis revealed that among female lifestyle variables, fertilization was negatively associated with smoke years (P=0.0003) and caffeine consumption (P=0.0037) (Table 2). Among male lifestyle variables, fertilization was negatively associated with caffeine consumption (P=0.0309) and IVF stress (P=0.0005) **(Table 2).**

Gender	Variable	Deviance	df	Deviance change	[a]	p value
Female	Smoke Years	355.5	150	13.21	-0.028	0.0003
	Caffeine (mg/wk)	360.3	150	8.4	-0.343×10^{-4}	0.0037
	Alcohol(std drinks/wk)	368.7	150	0.05	-	0.8205
	Daily Stress	367.4	150	1.29	-	0.2570
	IVF Stress	365.8	150	2.88	-	0.0890
Male	Smoke Years	368.0	150	0.69	-	0.4046
	Caffeine (mg/wk)	364.1	150	4.64	-1.403×10^{-4}	0.0309
	Alcohol(std drinks/wk)	365.1	150	3.69	-	0.0613
	Daily Stress	366.2	150	2.52	-	0.1121
	IVF Stress	356.5	150	12.19	-0.015	0.0005

Table 2: Univariate analysis of Lifestyle Variables on Fertilization

In the multiple logistic regression model, female daily stress and male caffeine was present in 85% and 71% of the models generated. Based on the interpretation of these multiple logistic regression models, male IVF-related stress and male caffeine consumption had a detrimental effect on fertilization. Although male caffeine consumption was not significant as a main effect (P=0.2350), it did exert a measurable effect, as is demonstrated by the significant interaction between male caffeine and alcohol consumption (Table 3). This finding implied that male caffeine consumption negates, to some extent, the beneficial effect of male alcohol consumption on fertilization as reported previously [13]

Factor		SE	OR (95% CI)	p value
Constant	-0.016	0.441	-	0.9718
Female Age	-0.007	0.012	0.99 (0.97 - 1.02)	0.5470
Female Nicotine	-0.025	0.013	0.98 (0.95 - 1.01)	0.0559
Female Smoke Years/Age	-1.650	0.33	0.19 (0.10 - 0.28)	<0.0001
FemaleSmoke Years/Age*Nicotine	0.059	0.025	1.06 (1.01 - 1.1)	0.0187
Female Daily Stress	0.016	0.006	1.02 (1.01 - 1.03)	0.0059
Male Caffeine	0.120×10^{-3}	0.101×10^{-3}	1.00 (0.99 - 1.01)	0.2350
Male Alcohol	0.073	0.014	1.08 (1.05 - 1.11)	<0.0001
Male Fruit&Vegetable Consumption	0.032	0.007	1.03 (1.02 - 1.05)	<0.0001
Male IVF Stress	-0.020	0.005	0.98 (0.97 - 0.99)	<0.0001
Male Caffeine*Male Alcohol	-0.026×10^{-3}	0.008×10^{-3}	0.97 (0.96 - 0.99)	0.0007
Male Fruit & Veg*Male Alcohol	-0.001	0.516×10^{-3}	<1.00 (<1.00 - <1.00)	0.0144

Table 3: Final Logistic Regression Model of Factors on Fertilization

Lifestyle factors affecting pregnancy following IVF treatment as well as pregnancy loss were investigated. The couples who experienced a pregnancy loss before the 12th week of gestation were compared to couples whose pregnancy was ongoing at the 12th week. There was no association found between caffeine consumption and the chance of pregnancy nor the likelihood of first trimester pregnancy loss.

Discussion

Although this study showed that fertilization rates can be compromised, we did not show an association between caffeine consumption and the chance of pregnancy nor the rate of first trimester miscarriage. This undoubtedly relates to the IVF model where the average oocyte retrieval was around 10, still enabling a high chance of generating embryos for transfer. In the natural setting or when few oocytes are collected, this could translate to a reduced chance of pregnancy, although this was not explored in this study. Furthermore, the study period precedes the current era where blastocyst culture is preferred, leading to a high rate of single embryo transfers and more embryos cryopreserved, utilising vitrification. Current data from our IVF Centre shows that frozen embryo transfer cycles generate higher implantation rates than fresh IVF cycles and higher overall productivity per initiated cycle when live births are combined for fresh and frozen transfers [16]. Future caffeine studies should attend to these limitations and examine advanced criteria such as oocyte utilisation rate, embryo utilisation and livebirth productivity rates.

Following Curtin's findings, there have been two publications consisting of a systematic review from Denmark [17] and a population-based case-control study from Sweden[18] that have found an association between caffeine intake and spontaneous abortion. The Danish study found a significantly increased risk of spontaneous miscarriage with both 300mg and 600mg of caffeine consumption per day. The Swedish study concluded that there may be an increased risk of early miscarriage among non-smoking women carrying fetuses with normal karyotypes when moderate or high levels of caffeine were ingested. These studies may be more relevant for miscarriage risk having attended to confounders such as aneuploidy as well as analysing higher numbers by systematic review.

Notwithstanding the limitations, this study found that some lifestyle factors were significant predictors of fertilization rates in vitro. It showed that caffeine consumption was not significant as a main effect but, indirectly, it modified the beneficial effect of male alcohol consumption which was previously shown to be positively associated with fertilization [13]. The current analysis also revealed that male caffeine consumption did not interact with male fruit or vegetable consumption. Therefore, it was hypothesised that one of the beneficial effects of alcohol consumption is from the consumption of vitamin B12 (cyanocobalamin), either from supplementation or derived from Brewer's yeast for beer production, which is not shared with the beneficial effect of fruit and vegetable intake, and it is this effect that is likely negated by caffeine consumption. In recent years, an association has been demonstrated between caffeine, several B-vitamins and homocysteine levels [19]. This implies potential interference by caffeine in the One-Carbon metabolic pathway. Caffeine has been associated with increased plasma total homocysteine (tHcy), which has been shown to significantly reduce IVF outcomes such as implantation and pregnancy rate if left untreated [20].

It has been assumed that caffeine causes increased tHcy levels because its chemical structure is similar to that of theophylline, which acts as a vitamin B6 (Pyridoxine) antagonist [21,22,23]. Theophylline is also one of the metabolites of caffeine, and inhibits the enzyme pyridoxal kinase, which is a key enzyme in the conversion of vitamin B6 to its active form, pyridoxal-5'-phosphate, thus decreasing circulating vitamin B6 levels. Vitamin B6 deficiency causes increased tHcy levels, due to the significant role of vitamin B6 in the homocysteine trans-sulfuration metabolic pathway **(Figure 4)** [24].

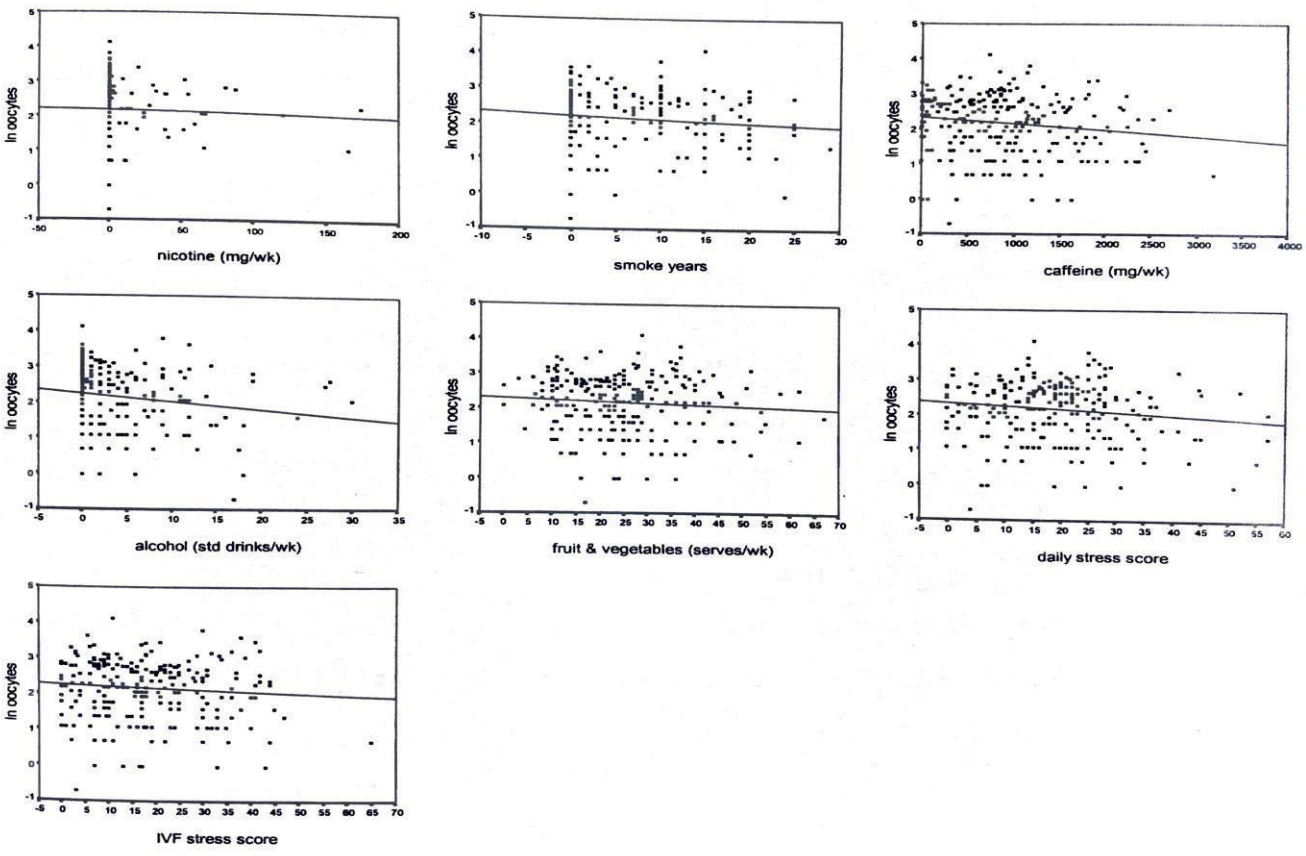

Figure 3: Scatterplots on ln(oocytes) and Lifestyle Variables

Another proposed mechanism by which caffeine causes raised tHcy levels is through the interaction between the hepatic metabolism of homocysteine and O-methylation of polyphenols [25]. Cholorogenic acid is the main phenolic compound in coffee [16], and methylation reactions transfer a methyl group from S-adenosylmethionine to polyphenols resulting in homocysteine **(Figure 5)** [26].

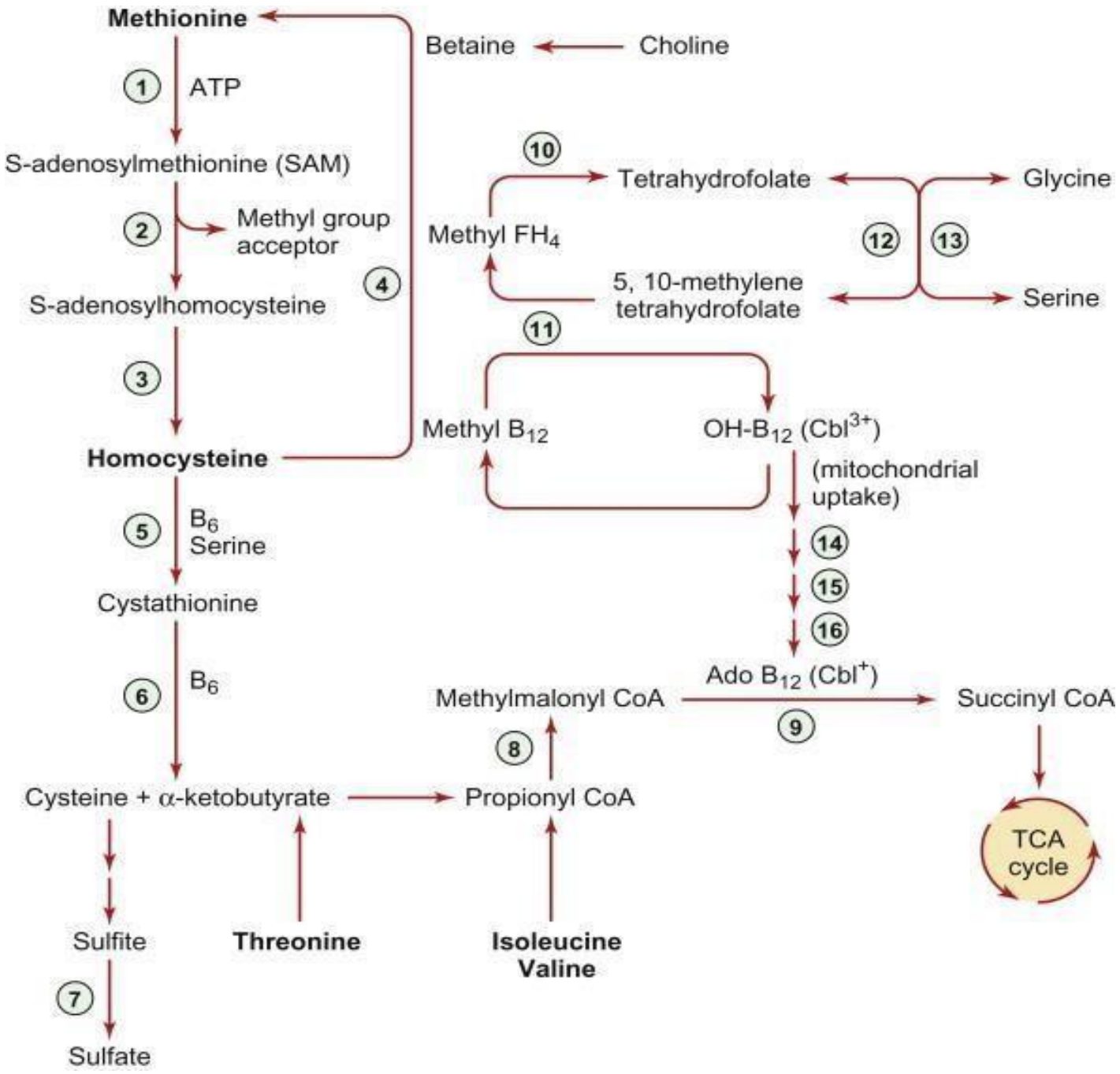

Figure 4: The trans-sulfuration pathway which is the major route for the metabolism of the sulfur- containing amino acids entails the transfer of the sulfur atom of methionine to serine to yield cysteine.

(Source: Basic Neurochemistry (8th edition). Principles of Molecular, Cellular, and Medical Neurobiology. 2012. Chapter 42: Disorder of Amino Acid metabolism, page 737-754).

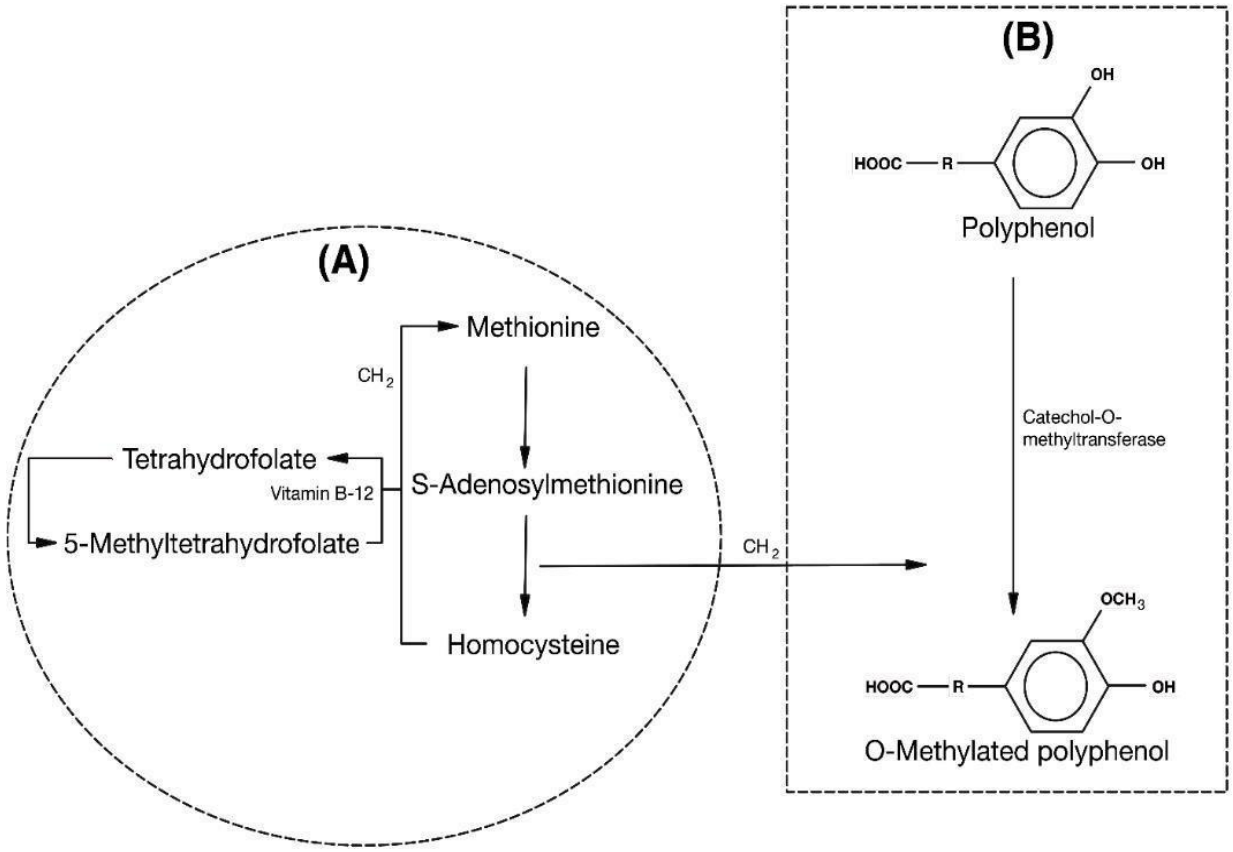

Figure 5: Proposed interaction between the hepatic metabolism of homocysteine (A) and the O-methylation of polyphenols (B).

It has been speculated therefore that consumption of high doses of polyphenols might increase homocysteine production through increased methylation reactions [27].

A 2008 cohort study of 10,601 healthy Norwegians found that there was a dose-dependent decrease in vitamin B concentrations with increasing coffee consumption for all vitamins except cobalamin [16]. This was thought to be from the effect caffeine had on the proximal renal tubules where most of the vitamins were reabsorbed causing increased vitamin excretion at high blood vitamin concentrations. The study concluded that coffee consumption was associated with reduced plasma concentration of folate, pyridoxal phosphate (B6) and riboflavin (B2), but mainly at high vitamin concentrations and it was therefore hypothesised that this was due to a loss of surplus B-vitamins through excretion in urine.

Additionally, caffeine consumption may affect ART outcome through caffeine's relationship with sirtuins. Sirtuins, an NAD(+)-dependent class III histone deacetylase (HDAC) protein, has been implicated as a protective factor against oxidative damage caused by stress related to aging and in vitro culture [28]. Caffeine acts to modulate intracellular sirtuin activity but is heavily influenced by dietary fat consumption [29]. In obese and overweight individuals in particular, excess caffeine consumption should be avoided due to the possibility of impaired liver pharmacokinetics.

Around the time of the Curtin study (1997-1998), there was only one other cohort study looking into the effects of caffeine on ART outcome [30]. Klonoff-Cohen et al. conducted a cohort study involving 221 women attending seven fertility clinics in Southern California. They found that women with a regular caffeine intake of either 2-50 and >50mg/day had adjusted odds ratio (95% confidence interval) of not achieving a live birth of 3.1 [1.1-9.7] and 3.9 [1.3-11.6] respectively, compared with women consuming <2mg/d. The study suggested that caffeine intake should be minimised while undergoing IVF or gamete intrafallopian transfer (GIFT). Only 36% of women in the current study underwent GIFT but this technique has largely been phased out. Furthermore, the Californian study was performed during a time when the median number of embryos transferred in one ET event was 4, which is likely to be a significant confounder.

Since then, there have been a few more studies that have investigated the effects of caffeine specifically on ART outcomes. Two of these studies focused on the intake of caffeine during the year prior to infertility treatment. The EARTH study team from Boston [31] and a further study from Milan [32] both showed no association between low to moderate caffeine intake and ART outcomes. A further separate group from Boston [33] conducted a large prospective study with 2474 couples aiming to examine the association between caffeine consumption and IVF outcomes. The study reported no association between caffeine consumption and IVF outcomes echoing similar observations by a more recent Boston / Tel-Aviv study [34] and an earlier study from Riyadh, Saudia Arabia [4].

The Matchinger, 2017 study (median caffeine intake 142mg/d) found no association between preconception caffeine and number of total, mature, and fertilised oocytes, embryo quality measures, implantation, clinical pregnancy, or live birth [34]. However, Al-Saleh's 2010 study (median caffeine intake of 456mg/d) was also the first to report that caffeine can reach the follicular fluid and suggested evidence of its possible harmful role on the subsequent reproductive processes [4].

A recent, 2017 report from the EARTH study group [35] appears to support this current study. They found a positive association between male partner's alcohol intake and probability of achieving live birth as a result of ART. Conversely, caffeine intake was associated with a lower probability of achieving live birth after ART, but it may be important to recognise that these findings were limited to ICSI cycles. The study concluded that male pre-treatment caffeine and alcohol intakes were associated with live birth after ART but notwith semen parameters. This could be attributed to caffeine's action ofinhibition of phosphodiesterase (PDE) enzyme activity.

As a methylxanthine, caffeine is classified as a nonspecific PDE inhibitor, a group which also includes pentoxifylline (PF) and theophylline [36]. Methylxanthines cause an increase in intracellular cAMP and this has been shown to be beneficial by stimulating sperm motility in males with oligo-asthenospermia e.g. using PF at low to moderate doses in vitro for IVF sperm preparations [37]. However, very high doses of PF have been shown to cause sperm DNA fragmentation and lower live birth rate with IVF. Interestingly, it has been shown that there is a difference in the action of caffeine at the site of in vitro fertilisation between cattle and swine. A study from Japan concluded that for insemination with bovine IVF, caffeine-free fertilisation medium is recommended, while caffeine remains essential in the fertilisation medium for successful porcine IVF [38]. Furthermore, in using PF for sperm enhancement, it is imperative to wash the PF out after 30-40 minutes incubation. This step not only provides optimum sperm enhancement but also avoids any contamination of oocytes as elevated cAMP inhibits germinal vesicle breakdown and further oocyte maturation including polar body release [37].

A recent systematic review of dietary effects on male fertility has found that high caffeine intake negatively impacts male fertility in vivo and in vitro [39]. In addition, the authors from another Japanese study theorised that, due to caffeine's very similar structure to adenosine, it could act as an adenosine antagonist. This might disrupt ATP-related energy metabolism, a process which is necessary for RNA and DNA synthesis, and thereby adversely impact on spermatogenesis [40]. By injecting male mice with the equivalent of 300mg to 1500mg of caffeine a day prior to sperm collection, they demonstrated a significant reduction in blastocyst formation rate, a finding which supports their hypothesis.

Although the current Curtin study was completed more than 15 years ago, the amount of data and information obtained remains invaluable, as there is still no definitive answer regarding an association between caffeine consumption and ART outcomes. Among the strengths of the Curtin study was that it accounted for caffeine consumed from coffee, tea, kola and iced coffee beverages. The diary devised by the Curtin group also took account of decaffeinated beverages, brewing methods of coffee, the proportion of the beverage consumed, and caffeine-containing prescription and 'over-the-counter' medication.

Ultimately, the Curtin findings in addition to more recent reports suggest that caffeine consumption does play a part in influencing the outcome of ART treatment possibly through its effects on inhibition of PDE as well as increasing tHcy levels. However, these effects are associated with high doses of caffeine, which suggested that there is an acceptable amount of caffeine that can be consumed.

Caffeine consumption has been classified as low users (<200mg/day), moderate users (200-400mg/day) and high users (>400mg/day) [5]. Caffeine intake at high doses, exceeding 500-600mg (equivalent to 4-7 cups/day) can cause anxiety, tremor and tachycardia but generally, life-threatening caffeine overdoses have been attributed to ingestion of caffeine-containing medications rather than caffeinated foods or beverages. The caffeine lethal dose has been estimated to be roughly 140-170mg/kg, which is equivalent to 8-10g/day (approximately 100 cups of coffee) [41].

The current guideline from the American College of Obstetricians and Gynaecologists suggests pregnant women and those capable of pregnancy to limit their caffeine intake to <200mg/d [42]. This seems to be a reasonable recommendation to adopt for those undertaking ART until further definitive information is available. Future studies should examine those components of the one-Carbon metabolic pathway which could interact with caffeine. This means examining the concomitant nutritional intake of foods containing B-group vitamins, particularly thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), folic acid (B9) and cyanocobalamin (B12). The measurement of tHcy levels will also be relevant as an indirect measure of deficiency or interference in one-Carbon metabolism, the importance of which is well established in a wide range of reproductive functions.

Authors' contributions

JLY supervised both this work and co-supervised, with AHL, the earlier PhD studies for KAJ. Data was initially collected by KAJ and statistically analysed by KAJ and AHL. SZ created the first draft of this manuscript and re-analysed the data. The manuscript was critically revised by JLY. All authors read and approved the final manuscript which was completed by JLY.

References:

1. Nawrot, P. Jordan, S. Eastwood, J. Rotstein, J.Hugenholtz, A. et al. (2003) Effects of caffeine on human health. Food Additives and Contaminants. 20, 1-30.
2. Berger, (1988) A. Effects of caffeine consumption on pregnancy outcome. A review. Journal of Reproductive Medicine. 33, 945–956.
3. Arnaud, M.J. (1999) Caffeine: chemistry and physiological effects. Encyclopedia of Human Nutrition, edited by Sadler, M.J. Stain, J.J.Caballero, B. San Diego: Academic Press. 206– 214.
4. Al-Saleh, I. El-Doush, I.Grisellhi, B. Coskun, (2010) S.The effect of caffeine consumption on the success rate of pregnancy as well various performance parameters of in-vitro fertilization treatment. Med. Sci. Monit.16, 598-605.
5. Capelletti, S. Daria, P. Sani, G. Aromatario, M. (2015) Caffeine: Cognitive and Physical Performance Enhancer or Psychoactive Drug? Current Neuropharmacology. 13, 71-88.
6. Benowitz, N.L. (1990) Clinical pharmacology of caffeine. Annu Rev Med. 41, 277-278.
7. Arnaud, M.J. (2011) Pharmacokinetics and metabolism of natural methylxanthines in animal and man. Handb. Exp. Pharmacol. 33–91.
8. Grosso, L.M. Bracken, M.B. (2005) Caffeine metabolism, genetics, and perinatal outcomes: a review of exposure assessment considerations during pregnancy. Ann. Epidemiol. 15, 460– 466.
9. Graham, T.E. (2001) Caffeine and exercise: metabolism, endurance and performance. Sport. Med. 31, 785–807.
10. Yang, A. Palmer, A.A. De Wit, H. (2010) Genetics of caffeine consumption and responses to caffeine. Psychopharmacology (Berl). 211, 245–257.
11. Joesbury, K.A. (2003) The Impact of Lifestyle Factors on the Clinical Outcomes of in vitro Fertilization-Embryo Transfer (IVF) Treatment. Curtin University of Technology, Perth, Australia.
12. Joesbury, K.A. Edirisinghe, W.R. Phillips, M.R. Yovich, J.L. (1998) Evidence that male smoking affects the likelihood of a pregnancy following IVF treatment: application of the modified cumulative embryo score. Hum. Reprod. 13, 1506-1513.
13. Firns, S. Cruzat V.F. Keane, K.N. Joesbury, K.A. Lee, A.H.; Newsholme, P. Yovich, J.L. (2015) The effect of cigarette smoking, alcohol consumption and fruit and vegetable consumption on IVF outcomes: a review and presentation of original data. Reprod. Biol. Endocrinol. 13, 1-13.
14. Schreiber, G. Maffeo, C.; Robins, M. Masters, M. Bond, A. (1988) Measurement of coffee and caffeine intake: implications for epidemiological research. Preventive Medicine. 17, 280-294.
15. Caffeine Survey: Western Australian Food Monitoring Program (1995) Chemistry Centre (WA), Department of Minerals and Energy (WA) & Health Department of WA.
16. Yovich JL et al. (2019) Growth hormone adjuvant trial for poor responders undergoing IVF. Eur J Obstet Gynecol 236: 249-251
17. Lyngso J, Ramlau-Hansen CH, Bay B, Ingerslev HJ, Hulman A, Kesmodel US. (2017) Association between coffee or caffeine consumption and fecundity and fertility: a

systematic review and dose-response meta-analysis. Clin Epidemiol 9: 699-719
18. Cnattingius S, Signorello LB, Anneren G, Clausson B, Ekbom A. et al (2000) Caffeine intake and the risk of first trimester spontaneous abortion. N Engl J Med 21; 343 (25): 1839-1845
19. Ulvik, A. Vollset, S.E. Hoff, G. Ueland, P.M. (2008) Coffee Consumption and circulating B- Vitamins in Healthy Middle-Aged Men and Women. Clinical Chemistry. 54 (9), 1489-1496.
20. Pacchiarotti, A. Mohamed, M.A. Micara, G. Linari, A. Tranquilli, D. Espinola, S.B. Aragona, C.(2007) The possible role of hyperhomocysteinemia on IVF outcome. J Assist Reprod Genet. 24 (10), 459-462.
21. Shiraishi, M. Haruna, M. Matsuzaki, M. Ota, E. Murayama, R. Sasaki, S.Yeo, S.A. Murashima, S. (2014) Relationship between plasma total homocysteine level and dietary caffeine and vitamin B6 intakes in pregnant women. Nurs Health Sci. 16(2), 164-170.
22. Ubbink, J.B.; Delport, R.; Becker, P.J.; Bissbort, S. (1989) Evidence of a theophylline-induced vitamin B6 deficiency caused by non-competitive inhibition of pyridoxal kinase. J. Lab. Clin. Med. 113, 15–22.
23. Ubbink, J.B. Delport, R. Bissbort, S. Vermaak, W.J. Becker, P.J. (1990) Relationship between vitamin B-6 status and elevated pyridoxal kinase levels induced by theophylline therapy in humans. J. Nutr. 120, 1352– 1359.
24. Langman, L.J. Cole, D.E. (1999) Homocysteine. Crit. Rev. Clin. Lab. Sci. 36, 365– 406.
25. Olthof, M.R.Hollman, P.C.Zock, P.L. Katan, M.B. (2001) Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. Am J Clin Nutr. 73 (3), 532-538.
26. Finkelstein, J.D. (1990) Methionine metabolism in mammals. J Nutr Biochem. 1, 228-237.
27. Zhu, B.T. Ezell, E.L. Liehr, J.G. (1994) Catechol-O-methyltransferase-catalyzed rapid O- methylation of mutagenic flavonoids. Metabolic inactivation as a possible reason for their lack of carcinogenicity in vivo. J Biol Chem. 269, 292-299.
28. Tatone C, Di Emidio G, Vitti M, Di Carlo M, Santini Jr S. et al (2015) functions in female fertility: Possible role in oxidative stress and aging. Oxid Med Cell Longev, 659687
29. Martins IJ. (2017) Caffeine with links to NAFLD and accelerated brain aging. Chapter: Non-alcoholic Fatty Liver Disease - Molecular Bases, Prevention and Treatment. InTech - Open Science Open Minds.
30. Klonoff-Cohen, H. Bleha, J. Lam-Kruglick, P. (2002) A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-fallopian transfer. Hum. Reprod. 17, 1746-1754.
31. Abadia, L. Chiu, Y.H. Williams, P.L. Toth, T.L. Souter, I. Hauser, R. Chavarro, J.E. Gaskins, A.J. (2017) The association between pre-treatment maternal alcohol and caffeine intake and outcomes of assisted reproduction in a prospectively followed cohort. Hum. Reprod. 32, 1846-1854.
32. Ricci, E. Noli, S. Cipriani, S. La Vecchia, I. Chiaffarino, F. Ferrari, S. Mauri, P.A. Reschini, M. Fedele, L. Parazzini, F. (2018) Maternal and Paternal Caffeine Intake and ART Outcomes in Couples Referring to an Italian Fertility Clinic: A Prospective Cohort. Nutrients. 10, 1116.
33. Choi, J.H. Ryan, L.M. Cramer, D.W. Hornstein, M.D. Missmer, S.A. (2011) Effects of caffeine consumption by women and men on the outcome of in vitro fertilization. J. Caffeine Res. 1, 29-34.
34. Machtinger, R. Gaskins, A.J. Mansur, A. Adir, M. Racowsky, C. Baccarelli, A. Hauser, R. Chavarro, J.E. (2017) Association between preconception maternal beverage intake and in vitro fertilization outcomes. Fertil. Steril. 108, 1026-1033.
35. Karmon, A.E. Toth, T.L. Chiu, Y.H. Gaskins, A.J. Tanrikut, C. Wright, D.L. Hauser, R. Chavarro, J.E. (2017) Earth Study Team Male caffeine and alcohol intake in relation to semen parameters and in vitro fertilization outcomesamong fertility patients. Andrology 5, 354-361.
36. Drobnis, E.Z. Nangia, A.K. (2017) Impacts of medication on Male Fertility. Chapter 5: Phosphodiesterase Inhibitors (PDE Inhibitors) and Male Reproduction.
37. Yovich JL. Pentoxifylline: (1993) Actions and applications in assisted reproduction. Hum Reprod 8: 1786-1791.
38. Momozawa K, Fukuda Y. (2003) Caffeine in fertilization medium is not essential for bovine IVF by fully capacitated spermatozoa. J Reprod Dev, 49 (6): 507-512.
39. Salas-Huetos, A. Bullo, M. Salas-Salvado, J. (2017) Dietary patterns, food and nutrients in male fertility parameters and fecund ability: a systematic review of observational studies. Hum Reprod Update. 23 (4), 371-389.
40. Yokota Y, Yokota H, Yokota M, Araki Y, Araki Y. (2015) In Vitro fertilisation embryo development from caffeine-treated murine sperm. Reproductive Medicine and Biology ;14 (3): 117-121
41. Alsabri, S.G. Mari, W.O. Younes, S. Alsadawi, M.A. Oroszi, T.L. (2018) Kinetic and Dynamic Description of Caffeine. J. Caffeine Adenosine Res. 8, 3–9.
42. Committee Opinion No. 462. (2010) American College of Obstetricians and Gynecologists. Moderate caffeine consumption during pregnancy. Obstet Gynecol. 116, 467–468.