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Is there an association between artificial sweetener consumption and in-vitro reproduction outcomes?

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KEY MESSAGE

Women consuming regular or diet soft drinks are at increased risk of oocyte dimorphisms, diminished embryo quality and a mild negative effect on blastocyst formation, implantation and pregnancy rates. Unfavourable embryo development was observed in women consuming artificially sweetened coffee.

A B S T R A C T

Previous studies have suggested an association between high intake of sweetened beverages and a number of adverse health outcomes. In this cross-sectional study, we investigated the association between daily consumption of sweetened soft drinks or coffee and the outcome of intracytoplasmic sperm injection (ICSI) treatment. Patients (n = 524) were interviewed by a nutritionist before ICSI treatment, using a food frequency questionnaire. Regression analysis showed that consumption of ≥3 servings of regular soft drinks or any amount of diet soft drinks was associated with oocyte dysmorphism, diminished embryo quality on days 2 and 3 of culture, and a mild effect on blastocyst formation, implantation and pregnancy rate. Consumption of artificially sweetened coffee was negatively associated with embryo quality on days 2 and 3. However, consumption of coffee or soft drinks was not associated with the odds of live birth. Even so, patients should be advised about the potential negative effects of sugar and artificial sweeteners before attempting infertility treatment. This study is limited by the use of a non-validated food frequency questionnaire, lack of information on quantity of sweeteners consumed, and lack of data on glucose levels in blood serum or follicular fluid. Further investigation is warranted.

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https://doi.org/10.1016/j.rbmo.2017.11.004
1472-6483/© 2017 Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.

Please cite this article in press as: Amanda Souza Setti, Daniela Paes de Almeida Ferreira Braga, Gabriela Halpern, Rita de Cássia S. Figueira, Assumpto IaconelliJr, Edson BorgesJr, Is there an association between artificial sweetener consumption and in-vitro reproduction outcomes?, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.11.004

Introduction

Obesity has become a worldwide epidemic that has proven to be strongly associated with sugar intake (Sylvetsky et al., 2012). Probably due to the drastic increased prevalence of overweight and obesity, the consumption of sweeteners has sharply risen over recent decades (Ng and Popkin, 2012; Piernas et al., 2013; Spencer et al., 2016). Sweeteners, also known as low- or no-calorie sweetener, non-nutritive sweetener or artificial sweetener (Serván et al., 2014), is the term used to describe additives that provide sweetness without contributing to caloric intake (Blackburn et al., 1997; Chattopadhyay et al., 2014).

Although the potential risks of each sweetener are assessed before their approval (Olivier et al., 2015), the introduction of sweeteners onto the public market in the 1950s and 1960s has been accompanied by debates and disagreements regarding their potential nutritional and general health impacts (Rogers et al., 2016; Serván et al., 2014). Nonetheless, at present these compounds are used throughout the world in the formulation of reduced-calorie beverages and foods, and medicines (Olivier et al., 2015; Serván et al., 2014).

Soft drinks are the main sources of artificial sweeteners (Halldorsson et al., 2010; Magnuson, 2010). These beverages are often promoted as a better alternative to sugar-sweetened soft drinks, which are considered the main caloric contributor in the US diet (Block, 2004). Previous studies have suggested that both artificially sweetened soft drinks and sugar-sweetened soft drinks are positively associated with hypertension (Winkelmayer et al., 2005), metabolic syndrome (Dhingra et al., 2007; Lutsey et al., 2008) and type 2 diabetes (Schulze et al., 2004).

Considering that the human fertility rate has declined over time, it could be argued that eating habits, including the consumption of sugar and artificial sweeteners, may negatively contribute to fertility potential. The effects of nutrition on the success of intracytoplasmic sperm injection (ICSI) have previously been explored. It has been demonstrated that female obesity negatively influenced the fertilization rate and the odds of miscarriage (Ferreira et al., 2010). Moreover, a positive association between the intake of artificially sweetened soft drinks and the risk of pre-term delivery has been previously demonstrated in two epidemiological studies (Englund-Ogge et al., 2012; Halldorsson et al., 2010).

To date, the association between the consumption of sweeteners and human assisted reproduction has not been investigated. The aim of this study was to evaluate whether the oocyte quality and ICSI outcomes are influenced by the daily consumption of soft drinks or coffee, sweetened with sugar or artificial sweeteners.

Materials and methods

Study design

This retrospective cross-sectional study included 5548 oocytes retrieved from 524 patients undergoing ICSI cycles between January 2012 and December 2014.

All patients completed a questionnaire with multiple-choice questions before treatment started. Women were asked about the frequency of consumption of many food items, including regular and diet soft drinks, unsweetened coffee and coffee sweetened with sugar or any kind of artificial sweetener.

The effects of dietary habits on the oocyte quality, embryo quality on day 2 and 3, chances of blastocyst formation, pregnancy, implantation and miscarriage rates were investigated. In order to avoid any influence of seminal parameters on the results, only couples undergoing ICSI as a result of female or unexplained infertility were included in this study.

A written informed consent was obtained in which patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by the local Institutional Review Board (protocol 410/2012) on 19 December 2012.

Food consumption questionnaire

All patients were interviewed face-to-face by the same nutrition professional, with special skills in dietary assessment methods, using a non-validated food frequency questionnaire, before the beginning of the ICSI treatment. The food frequency questionnaire is a subjective measure using a predefined, interviewer-administered format, in which data are collected based on usual intake estimates over a relatively long period (e.g. 6 months or 1 year) (Shim et al., 2014).

The questionnaire contained multiple-choice questions about the average frequency of consumption of food items during the past year. The food categories investigated were (i) regular soft drinks, (ii) diet soft drinks, (iii) unsweetened coffee, (iv) coffee with sugar and (v) coffee with artificial sweetener.

In the questionnaire, participants were asked to answer 'yes' or 'no' to the following questions:

1. Do you consume coffee daily?

If you answered 'yes', please answer how do you ingest your coffee:

unsweetened;
sweetened with sugar;
sweetened with any kind of artificial sweetener.

If you answered 'yes', please answer the number of servings (a 240 mL cup = 1 serving) you ingest per day:

1 serving per day;
2 servings per day;
3 or more servings per day.

2. Do you consume soft drinks daily?

If you answered 'yes', please answer what kind of soft drink you ingest:

regular;
diet or light.

If you answered 'yes', please answer the number of servings (a 240 mL cup = 1 serving) you ingest per day:

1 serving per day;
2 servings per day;
3 or more servings per day.

Controlled ovarian stimulation

A controlled ovarian stimulation was achieved by using recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) for ovarian

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stimulation and a gonadotrophin-releasing hormone (GnRH) antagonist (Cetrotide; Serono, Geneva, Switzerland) for pituitary blockage. The follicular growth was monitored using transvaginal ultrasound examination starting on day 4 of gonadotrophin administration. When adequate follicular growth and serum oestradiol levels were observed, recombinant human chorionic gonadotrophin (HCG) (Ovidrel; Serono, Geneva, Switzerland) was administered to trigger the final follicular maturation. The oocytes were collected 35 h after HCG administration through transvaginal ovarian puncture.

Preparation of oocytes

Retrieved oocytes were maintained in culture media (Global® for fertilization, LifeGlobal, Connecticut, USA) supplemented with 10% protein supplement (LGPS, LifeGlobal) and covered with paraffin oil (Paraffin Oil P.G., LifeGlobal) for 2–3 h before removal of cumulus cells. The surrounding cumulus cells were removed after exposure to a HEPES-buffered medium containing hyaluronidase (80 IU/ml, LifeGlobal). The remaining cumulus cells were mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, USA).

Oocyte morphology was assessed using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon®, Tokyo, Japan) with a Hoffmann modulation contrast system under 400 × magnification, just before sperm injection (4 h after retrieval). The following oocyte dysmorphisms were recorded: (i) cytoplasmic central granulation; (ii) cytoplasmic colour; (iii) vacuoles in the ooplasm; (iv) smooth endoplasmic reticulum clusters (SERc) in the ooplasm; (v) large perivitelline space (PVS); (vi) PVS granularity; (vii) fragmented polar body (PB); (viii) zona pellucida (ZP) abnormalities; and (ix) shape abnormalities. Oocytes that were observed to have released the first PB were considered mature and were used for ICSI.

Intracytoplasmic sperm injection

ICSI was performed in a micro-injection dish prepared with 4 µl droplets of buffered medium (Global® w/HEPES, LifeGlobal) and covered with paraffin oil on a heated stage at 37.0 ± 0.5°C of an inverted microscope. Approximately 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second PB. Embryos were maintained in a 50 µl drop of culture medium (Global®, LifeGlobal) supplemented with 10% protein supplement covered with paraffin oil in a humidified atmosphere under 6% CO_2 at 37°C for 3 days.

Embryo quality and transfer

The embryo morphology was assessed 16–18 h post-ICSI and on the mornings of days 2, 3 and 5 of embryo development using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon, Tokyo, Japan) with a Hoffmann modulation contrast system under 400 × magnification.

High-quality embryos were defined as those with 4 cells on day 2 or 8 – 10 cells on day 3, <15% fragmentation, symmetric and mononucleated blastomeres, and absence of cytoplasmic inclusions and/or dysmorphisms in the perivitelline space and ZP.

On day 5 of development, embryos that reached the blastocyst stage were considered when: (i) the blastocoel was greater than half the volume of the embryo; (ii) the blastocoel completely filled the embryo; (iii) the blastocyst was expanded; (iv) blastocyst hatching occurred; and (v) blastocyst hatched.

All of the embryo transfers were performed on day 5 of embryo development with the use of a soft catheter with transabdominal ultrasound guidance. Two to three embryos were transferred per patient.

Clinical follow-up

A pregnancy test was performed 12 days after embryo transfer. All women with a positive test had a transvaginal ultrasound scan 2 weeks after the positive test. A clinical pregnancy was diagnosed when the fetal heartbeat was detected. Pregnancy rates were calculated per transfer. Miscarriage was defined as clinical pregnancy loss before 20 weeks. The live birth rate was calculated per transfer.

Statistical analyses

The association between the consumption of regular and diet soft drinks, unsweetened coffee, and coffee with sugar or artificial sweetener on dichotomous variables such as (i) oocyte quality, (ii) embryo quality on cleavage stage, (iii) blastocyst formation, (iv) clinical pregnancy and (v) miscarriage chances were investigated using binary regression analyses, whereas linear regression analyses were conducted to evaluate the influence on (vi) implantation rate (continuous variable).

For statistical purposes, the consumption of regular and diet soft drinks, unsweetened coffee, and coffee with sugar or artificial sweetener were treated as independent variables. For all the independent variables, we only included in the analysis the daily consumers and the non-consumers. All other kinds of consumption were excluded from the analysis (e.g. patients reporting twice a week or every other day consumption). Daily consumption was considered any kind of consumption but none. Independent variables were coded as 0 (non-consumption) and 1 (daily consumption) in the binary regression analysis. Oocytes free of any morphological defect were considered high-quality oocytes, while oocytes presenting at least one morphological defect were considered low-quality oocytes. Oocyte quality was treated as dependent variable, and coded as 0 (low-quality oocyte) or 1 (high-quality oocyte) in the binary regression analysis. Embryos presenting the morphological characteristics cited above were considered high-quality embryos, while embryos presenting any morphological defect were considered of low quality. Embryo quality was treated as dependent variable, and coded as 0 (low-quality embryo) or 1 (high-quality embryo) in the binary regression analysis. Blastocyst formation was treated as dependent variable, and coded as 0 (embryos not reaching blastocyst stage) or 1 (embryos reaching blastocyst stage) in the binary regression analysis. Clinical pregnancy was treated as dependent variable, and coded as 0 (non-pregnant patient) or 1 (pregnant patient). Miscarriage was treated as dependent variable, and coded as 0 (ongoing pregnancy) or 1 (miscarriage). In this way, each patient acted as their own control.

All regression analyses were adjusted for maternal age, number of retrieved oocytes, maternal weight, smoking habit and physical activity, as these would be considered potential confounders of the association between the factors evaluated and the ICSI outcomes. Results were expressed as adjusted odds ratios (aOR) with 95% **confidence** interval (CI), or regression coefficients (RC) and *P*-value. Results were considered significant at the 5% critical level ($P \leq 0.05$). Data analysis was carried out using the Minitab (version 16) Statistical Program (Minitab, Pennsylvania, USA).

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Results

Descriptive analysis

Patients

Mean female age was 36.4 ± 5.0 years, mean body weight was 66.6 ± 12.0 kg, mean height was 1.6 ± 0.1 m, and mean body mass index (BMI) was 24.8 ± 4.3 kg/m^2.

Mean male age was 37.5 ± 5.8 years (range: 21–58), mean semen volume was 3.3 ± 1.4 ml (range: 1.5–12.8), mean sperm concentration per ml was 75.3 ± 48.5 × 10^6 (range: 15.0–505.0), mean total sperm concentration was 239.8 ± 177.9 × 10^6 (range: 39.0–2048.0), mean sperm motility was 64.2 ± 10.7% (range: 40.0–92.0), mean progressive sperm motility was 56.3 ± 12.2% (range: 32.0–91.0), and mean percentage of normal sperm forms was 4.7 ± 0.9% (range: 4.0–6.0).

Consumption of soft drinks and coffee

From 524 female patients, 354 reported a daily consumption of coffee (67.6%). Of those, 62 consumed unsweetened coffee (13 consumed 1 cup per day, 24 consumed 2 cups per day and 25 consumed ≥3 cups per day), 111 consumed coffee with sugar (30 consumed 1 cup per day, 49 consumed 2 cups per day and 32 consumed ≥3 cups per day) and 181 consumed coffee with artificial sweetener (57 consumed 1 cup per day, 59 consumed 2 cups per day and 65 consumed ≥3 cups per day). The daily consumption of soft drinks was reported by 258 patients (49.2%). Of those, 157 consumed regular soft drinks (82 consumed 1 cup per day, 54 consumed 2 cups per day and 21 consumed ≥3 cups per day) and 101 diet soft drinks (38 consumed 1 cup per day, 28 consumed 2 cups per day and 35 consumed ≥3 cups per day).

Ovarian stimulation

Mean total dose of FSH administered was 2214 ± 631 IU, number of follicles was 13.4 ± 12.4, number of obtained oocytes 9.7 ± 9.6, number of mature oocytes 7.4 ± 7.0, and mature oocyte rate 75.3 ± 20.7%.

Oocyte morphology

From the 4264 injected oocytes, 588 were morphologically normal (13.8%) and 3676 showed at least one morphological abnormality (86.2%). Central granulation in the ooplasm was observed in 62 oocytes (1.5%), vacuoles in 204 (4.8%), SERc in 120 (2.8%), large PVS in 742 (17.4%), PVS granularity in 2164 (50.8%), fragmented PB in 1260 (29.5%), ZP abnormalities in 596 (14.0%) and shape abnormalities in 216 (5.1%).

Intracytoplasmic sperm injection

ICSI outcomes are described in **Table 1**.

Table 1 – ICSI outcomes (*n* = 524).

Variables	Values
Injected oocytes	8.0 ± 6.3
Fertilization rate	75.2 ± 25.1
Embryos	6.8 ± 5.6
High-quality embryos	2.3 ± 3.2
Day 2 (%)	1770/3192 (55.5)
Day 3 (%)	1858/3192 (58.2)
Blastocyst formation (%)	518/1136 (45.6)
Embryos transferred	2.1 ± 1.0
Non-transferred cycles (%)	36/524 (6.9)
Clinical pregnancy rate (%)	155/488 (31.8)
Implantation rate (%)	231/1025 (22.5)
Miscarriage rate (%)	26/155 (16.8)
Pregnancy loss (after 20 weeks) rate	9/155 (5.8)
Ectopic pregnancy rate	5/155 (3.2)
Live birth rate (%)	115/488 (23.6)

Values are mean ± SD, unless otherwise noted.

Association between the consumption of coffee and soft drinks on oocyte quality

The influences of coffee and soft drink consumption on oocyte quality are shown in **Tables 2 and 3**, respectively. The consumption of unsweetened coffee or coffee sweetened with either sugar or artificial sweetener did not influence the occurrence of any oocyte dysmorphism, irrespective of the amount ingested per day (**Table 2**).

The consumption of ≥3 servings of regular soft drinks was associated with occurrence of central granulation (OR: 0.17, 95% CI: 0.12–0.23), vacuole (OR: 0.30, 95% CI: 0.22–0.40) and SERc (OR: 0.22, 95% CI: 0.17–0.29) (**Table 3**).

The consumption of any amount of diet soft drinks was also associated with occurrence of central granulation, vacuole and SERc. The odds ratios for high oocyte quality decreased with increased intakes for central granulation (1 serving per day: 0.34, 95% CI: 0.20–0.58, 2 servings per day: 0.07, 95% CI: 0.03–0.15; and ≥3 servings per day: 0.01, 95% CI: 0.00–0.07), vacuole (1 serving per day: 0.39, 95% CI: 0.26–0.58, 2 servings per day: 0.38, 95% CI: 0.26–0.57; and ≥3 servings per day: 0.26, 95% CI: 0.16–0.41) and SERc (1 serving per day: 0.55, 95% CI: 0.34–0.90, 2 servings per day: 0.48, 95% CI: 0.29–0.77; and ≥3 servings per day: 0.36, 95% CI: 0.22–0.59) (**Table 3**).

Association between the consumption of soft drinks and coffee on embryo quality, and on ICSI outcomes

The influences of coffee and soft drink consumption on embryo quality and ICSI outcomes are shown in **Tables 4 and 5**, respectively.

The consumption of unsweetened coffee and coffee with sugar did not influence any evaluated parameter; however, when artificial sweetener was added, negative amount-dependent influences were observed on embryo quality on day 2 (1 serving per day: 0.65, 95% CI: 0.30–0.91, 2 servings per day: 0.56, 95% CI: 0.39–0.86; and ≥3 servings per day: 0.50, 95% CI: 0.32–0.89) and day 3 (1 serving per day: 0.68, 95% CI: 0.33–0.97, 2 servings per day: 0.59, 95% CI: 0.38–0.96; and ≥3 servings per day: 0.55, 95% CI: 0.39–0.97). The consumption of artificially sweetened coffee also tended to decrease blastocyst formation, implantation and pregnancy chance, in an amount-dependent manner. The consumption of coffee was not associated with the odds of live birth (**Table 4**).

The consumption of regular soft drinks did not influence any evaluated parameter, irrespective of the amount ingested per day. On the other hand, the consumption of any amount of artificially sweetened soft drinks was negatively associated with embryo quality on days 2 (1 serving per day: 0.71, 95% CI: 0.35–0.86, 2 servings per day: 0.55, 95% CI: 0.26–0.90; and ≥3 servings per day: 0.42, 95% CI: 0.23–0.93) and day 3 (1 serving per day: 0.92, 95% CI: 0.44–0.97, 2 servings per day: 0.73, 95% CI: 0.38–0.96; and ≥3 servings per day: 0.61, 95% CI: 0.25–0.87). While blastocyst formation, implantation rate and pregnancy chance were not associated with the consumption of 1 serving of diet soft drinks per day, negative dose-dependent influences were observed for the consumption of ≥2 servings per day (blastocyst

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Table 2 – Results of binary regression analysis for the association between the consumption of coffee and oocyte quality.

Oocyte morphology Coffee	Polar body	PVS size	PVS granularity	Zona pellucida	Shape	Central granulation	Vacuole	SERc
Unsweetened coffee								
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	2.31 (0.29–18.32)	1.25 (0.39–4.03)	2.54 (0.89–7.25)	0.61 (0.08–4.46)	1.25 (0.94–1.66)	1.25 (0.94–1.66)	2.05 (0.43–9.64)	0.72 (0.41–1.24)
2 servings a day	0.84 (0.43–1.63)	1.73 (0.78–3.82)	1.02 (0.99–1.05)	0.60 (0.26–1.39)	1.02 (0.65–1.61)	1.02 (0.96–1.09)	0.98 (0.90–1.07)	0.96 (0.87–1.05)
≥3 servings a day	1.01 (0.91–1.12)	1.45 (0.19–11.11)	1.00 (0.79–1.25)	0.96 (0.73–1.26)	0.76 (0.35–1.65)	1.03 (0.26–4.15)	0.34 (0.06–2.05)	0.51 (0.10–2.55)
Coffee with sugar								
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	0.76 (0.35–1.66)	0.97 (0.65–1.45)	0.74 (0.46–1.17)	1.12 (0.73 -1.72)	0.51 (0.28–1.21)	0.81 (0.64–1.23)	0.91 (0.53–1.77)	1.36 (0.77–2.39)
2 servings a day	0.27 (0.03–2.12)	0.78 (0.64–1.31)	2.46 (0.29–21.09)	1.49 (0.53–4.24)	1.15 (0.35–3.78)	0.70 (0.17–2.92)	1.88 (0.84–4.21)	1.36 (0.17–10.80)
≥3 servings a day	1.42 (0.92–2.18)	0.46 (0.16–1.32)	1.00 (0.45–2.19)	1.06 (0.55–2.02)	0.82 (0.59–1.14)	0.25 (0.05–1.39)	0.60 (0.31–1.15)	0.98 (0.55–3.84)
Coffee with artificial sweetener								
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	1.20 (0.85–1.71)	1.45 (0.40–5.21)	0.65 (0.27–1.55)	1.22 (0.72–2.06)	1.19 (0.87–1.62)	1.27 (0.71–2.29)	1.02 (0.75–1.39)	0.84 (0.57–1.23)
2 servings a day	1.10 (0.77–1.58)	1.32 (0.92–1.89)	1.07 (0.45–2.52)	1.10 (0.91–1.33)	1.12 (0.82–1.53)	1.29 (0.72–2.31)	0.98 (0.72–1.33)	0.80 (0.55–1.18)
≥3 servings a day	1.18 (0.83–1.69)	1.20 (0.45–2.76)	0.65 (0.27–1.55)	1.11 (0.92–1.35)	0.91 (0.68–1.23)	1.34 (0.75–2.41)	1.22 (0.88–1.69)	1.06 (0.70–1.59)

PVS = perivitelline space; SERc = smooth endoplasmic reticulum cluster.
Values are adjusted odds ratio with 95% confidence intervals. All P-values are non-significant.

Table 3 – Results of binary regression analysis for the association between the consumption of soft drinks and oocyte quality.

Oocyte morphology Soft drink	Polar body	PVS size	PVS granularity	Zona pellucida	Shape	Central granulation	Vacuole	SERc
Regular soft drink								
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	0.93 (0.80–1.07)	1.04 (0.88–1.24)	1.06 (0.93–1.21)	1.09 (0.91–1.30)	1.15 (0.85–1.55)	0.76 (0.40–1.44)	1.00 (0.88–1.52)	0.82 (0.60–1.19)
2 servings a day	0.92 (0.80–1.06)	0.92 (0.78–1.09)	1.01 (0.88–1.15)	1.12 (0.92–1.35)	1.05 (0.78–1.42)	0.73 (0.52–1.03)	0.84 (0.49–1.09)	0.69 (0.42–1.12)
≥3 servings a day	1.10 (0.96–1.27)	0.94 (0.80–1.12)	0.99 (0.86–1.13)	1.01 (0.84–1.22)	0.74 (0.52–1.24)	0.17 (0.12–0.23)[a]	0.30 (0.22–0.40)[e]	0.22 (0.17–0.29)[i]
Diet soft drink								
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	1.04 (0.87–1.24)	1.11 (0.88–1.39)	0.99 (0.74–1.11)	1.02 (0.90–1.24)	0.91 (0.70–1.13)	0.34 (0.20–0.58)[b]	0.39 (0.26–0.58)[f]	0.55 (0.34–0.90)[j]
2 servings a day	1.19 (0.79–1.42)	1.06 (0.85–1.33)	0.91 (0.82–1.05)	1.10 (0.88–1.31)	1.00 (0.74–1.02)	0.07 (0.03–0.15)[c]	0.38 (0.26–0.57)[g]	0.48 (0.29–0.77)[k]
≥3 servings a day	0.87 (0.73–1.03)	0.91 (0.74–1.13)	1.13 (0.69–1.28)	0.99 (0.81–1.16)	1.05 (0.89–1.30)	0.01 (0.00–0.07)[d]	0.26 (0.16–0.41)[h]	0.36 (0.22–0.59)[l]

Values are: adjusted odds ratio with 95% confidence intervals. Significant *P*-values: a = 0.036, b = 0.032, c = 0.036, d = 0.05, e = 0.045, f = 0.043, g = 0.025, h = 0.042, i = 0.049, j = 0.047, h = 0.031, i = 0.027.
PVS = perivitelline space; SERc = smooth endoplasmic reticulum cluster.

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Table 4 – Results of binary and linear regression analysis for the association between the consumption of coffee and ICSI outcomes.

ICSI outcomes Coffee	Embryo quality on day 2	Embryo quality on day 3	Blastocyst formation	Implantation rate	Clinical pregnancy	Miscarriage	Live birth
Unsweetened coffee							
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	1.08 (0.56–1.21)	0.98 (0.36–2.17)	1.03 (0.52–2.08)	RC: 2.514, r^2: 0.1%	1.01 (0.58–1.66)	1.06 (0.45–2.32)	1.04 (0.67–1.64)
2 servings a day	1.05 (0.51–1.16)	1.01 (0.54–1.77)	1.00 (0.49–1.82)	RC: 2.995, r^2: 0.15%	0.97 (0.55–1.74)	0.99 (0.29–2.01)	1.11 (0.71–1.73)
≥3 servings a day	1.11 (0.59–1.34)	1.10 (0.57–1.31)	1.04 (0.42–1.29)	RC: 2.416, r^2: 0.1%	1.00 (0.52–1.50)	0.98 (0.38–1.99)	1.09 (0.70–1.70)
Coffee with sugar							
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	0.53 (0.21–1.18)	0.87 (0.33–1.56)	0.98 (0.50–1.17)	RC: 2.566, r^2: 0.2	0.95 (0.46–1.80)	0.66 (0.32–1.20)	1.21 (0.78–1.87)
2 servings a day	0.61 (0.12–1.22)	0.89 (0.39–1.57)	1.05 (0.55–1.20)	RC: 2.805, r^2: 0.1	0.99 (0.45–1.77)	0.51 (0.26–1.19)	1.36 (0.88–2.10)
≥3 servings a day	0.60 (0.11–1.33)	0.81 (0.29–1.55)	1.03 (0.49–1.41)	RC: 3.914, r^2: 0.1	1.01 (0.49–1.71)	0.50 (0.24–1.15)	1.29 (0.81–2.00)
Coffee with artificial sweetener							
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	0.65 (0.30–0.91)[a]	0.68 (0.33–0.97)[c]	0.80 (0.40–1.01)	RC: −0.050, r^2: 4.1%	0.91 (0.70–1.02)	1.00 (0.41–2.45)	0.83 (0.54–1.28)
2 servings a day	0.56 (0.39–0.86)[b]	0.59 (0.38–0.96)[d]	0.79 (0.43–1.02)	RC: −0.106, r^2: 4.4%	0.92 (0.75–1.00)	1.01 (0.37–2.61)	0.78 (0.51–1.20)
≥3 servings a day	0.50 (0.32–0.89)[c]	0.55 (0.39–0.97)[e]	0.75 (0.65–1.00)	RC: −0.158, r^2: 5.5%	0.88 (0.73–1.10)	1.01 (0.36–2.64)	0.70 (0.46–1.08)

Values are adjusted odds ratio with 95% confidence intervals, unless otherwise noted.
RC = regression coefficient.
Significant *P*-values: a = 0.045, b = 0.021, c = 0.025, d = 0.05, e = 0.041.

Table 5 – Results of binary and linear regression analysis for the association between the consumption of soft drinks and ICSI outcomes.

ICSI outcomes Soft drink	Embryo quality on day 2	Embryo quality on day 3	Blastocyst formation	Implantation rate	Clinical pregnancy	Miscarriage	Live birth
Regular soft drink							
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	0.54 (0.10–1.66)	0.93 (0.47–1.67)	0.99 (0.40–2.17)	RC: 2.365, r^2: 0.1%, *P* = NS	1.01 (0.49–1.96)	0.75 (0.43–1.55)	0.90 (0.54–1.49)
2 servings a day	0.55 (0.11–1.27)	0.91 (0.51–1.89)	1.01 (0.52–2.14)	RC: 2.154, r^2: 0.1%, *P* = NS	0.99 (0.66–2.18)	1.22 (0.29–3.74)	0.88 (0.53–1.45)
≥3 servings a day	0.32 (0.19–1.30)	0.62 (0.30–1.25)	1.07 (0.46–1.99)	RC: 2.009, r^2: 0.1%, *P* = NS	1.03 (0.42–1.80)	0.99 (0.33–2.22)	0.83 (0.50–1.39)
Diet soft drink							
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1 serving a day	0.71 (0.35–0.86)[a]	0.92 (0.44–0.97)[d]	0.79 (0.66–1.05)	RC: −0.996, r^2: 3.5%, *P* = NS	0.82 (0.66–1.02)	1.06 (0.28–5.60)	0.81 (0.49–1.34)
2 servings a day	0.55 (0.26–0.90)[b]	0.73 (0.38–0.96)[e]	0.95 (0.50–0.99)[g]	RC: −1.452, r^2: 6.1%, *P* = 0.040	0.80 (0.48–0.95)[i]	1.51 (0.26–3.19)	0.70 (0.42–1.18)
≥3 servings a day	0.42 (0.23–0.93)[c]	0.61 (0.25–0.87)[f]	0.93 (0.49–0.99)[h]	RC: −1.958, r^2: 6.5%, *P* = 0.032	0.67 (0.35–0.94)[j]	1.62 (0.33–4.11)	0.65 (0.39–1.09)

Values are adjusted odds ratio with 95% confidence intervals, unless otherwise noted.
RC = regression coefficient; NS = non-significant.
Significant *P*-values: a = 0.050, b = 0.029, c = 0.042, d = 0.047, e = 0.027, f = 0.038, g = 0.049, h = 0.022, i < 0.01, j = 0.042.

formation [2 servings per day: 0.95, 95% CI: 0.50–0.99; and ≥3 servings per day: 0.93, 95% CI: 0.49–0.99]; implantation rate [2 servings per day: −1.452, r^2: 6.1%, $P = 0.040$; and ≥3 servings per day: −1.958, r^2: 6.5%, $P = 0.032$]; and pregnancy chance [2 servings per day: 0.80, 95% CI: 0.48–0.95; and ≥3 servings per day: 0.67, 95% CI: 0.35–0.94]). The consumption of soft drinks was not associated with the odds of live birth (**Table 5**).

Discussion

The aim of this study was to investigate possible associations between daily intake of soft drinks and coffee, sweetened with sugar or artificial sweeteners, and oocyte quality and ICSI outcomes. We observed increased risk of intracytoplasmic oocyte dysmorphisms in women consuming ≥3 servings of regular soft drinks or any amount of diet soft drinks. Unfavourable dose-dependent embryo development on days 2 and 3 was observed in women consuming artificially sweetened coffee or artificially sweetened soft drinks. Artificially sweetened coffee consumption also tended to decrease blastocyst formation, implantation and pregnancy chance. Negative dose-dependent influences were observed for the consumption of 2 and ≥3 servings per day of diet soft drinks on blastocyst formation, implantation rate and pregnancy chance.

Artificial sweeteners are widely used to substitute sugar in foods and beverages. This shift toward sugar replacement is likely to be a result of recent obesity-prevention campaigns and the emergent reputation of low-carbohydrate diets for weight loss (Rogers et al., 2016; Sylvetsky et al., 2012). Previous studies demonstrated that the overall intake of artificial sugar substitutes has been increasing over time (Mattes and Popkin, 2009; Sylvetsky et al., 2011). Beverages in general, mainly diet soft drinks, are ranked the most popular vehicles for artificial sweeteners (Halldorsson et al., 2010; Magnuson, 2010; Mattes and Popkin, 2009). Data from the National Health and Nutrition Examination Survey suggested that beverages containing artificial sweeteners are consumed on a daily basis by nearly 30% of adults in the USA (Sylvetsky et al., 2012).

Before their approval and introduction onto the public market, artificial sweeteners are subject to stringent safety assessments. One of those assessments is called the 'acceptable daily intake', which is a guarantee of safety that sets the quantity of a substance that can be consumed daily, over the lifespan, without any harmful effect on health (Serván et al., 2014). Nonetheless, a recent meta-analysis showed that the available data are insufficient to determine any long-term nutritional benefits related to the consumption of products containing artificial sweeteners as sugar substitutes. Additionally, it did not rule out potential long-term risks related to daily artificial sweetener intake (Olivier et al., 2015).

Although several interventional studies have shown that artificial sweeteners are effective for weight loss (Blackburn et al., 1997; Tate et al., 2012) and maintenance (Blackburn et al., 1997), there is also evidence that, regardless of their intrinsic lack of calories, artificial sweeteners may negatively influence glucose metabolism (Brown et al., 2009), satiety (Pepino and Bourne, 2011), vascular function (Gardener et al., 2012), and even increase the risk of becoming overweight and obese (Fowler et al., 2008; Ludwig, 2009; Swithers, 2013). In addition, the consumption of sugar-sweetened and artificially sweetened soft drinks was associated with cardio-metabolic disorders and long-term weight gain (Dhingra et al., 2007; Fowler et al., 2008; Lutsey et al., 2008; Nettleton et al., 2009; Winkelmayer et al., 2005).

Both obesity and plasma glucose concentrations, which are negatively influenced by the consumption of sugar and artificial sweeteners, are associated with inflammatory response and pre-term delivery (Andraweera et al., 2012; Scholl et al., 2001). Therefore, it could be argued that reproductive health is also at risk. However, very few studies have investigated the potential effects of artificial sweeteners on women's reproductive health. Two studies investigated the effects of soft drink intake in pre-term delivery. Halldorsson et al. (2010) conducted a prospective analysis of 59,334 Danish pregnant women and observed that the daily intake of artificially sweetened soft drinks may increase the risk of pre-term delivery. Additionally, a dose–effect relationship was observed, meaning that the risk of pre-term delivery was higher in the heaviest consumers of artificially sweetened beverages. However, no association was observed for sugar-sweetened soft drinks. Later, Englund-Ogge et al. (2012) replicated the aforementioned study in a prospective analysis of 60,761 Norwegian pregnant women and observed an increased risk of pre-term delivery in women who consumed ≥1 serving of artificially sweetened soft drinks per day.

Two hypotheses have emerged to explain such associations: (i) methanol exposure and (ii) elevated glucose concentrations. Aspartame is the most common artificial sweetener used in products from the major international brands (Halldorsson et al., 2010). It is important to highlight that all artificially sweetened soft drinks in Brazil contain aspartame. The metabolism of aspartame forms methanol, which is oxidized to formic acid, which is responsible for the toxicity of methanol (Englund-Ogge et al., 2012; Halldorsson et al., 2010). Previous animal studies have shown that exposure to methanol and formic acid decreases gestational length (Burbacher et al., 2004; Trocho et al., 1998) by affecting the fetal neuroendocrine system or maternal uterine environment (Halldorsson et al., 2010). In addition, embryo toxicity from formic acid has been reported in animal models (Brown-Woodman et al., 1995), at levels that are only mildly maternally toxic, leading to significant decreases in embryo developmental score (Andrews et al., 1998) and depletion of glutathione in the embryo (Hutson et al., 2013). Therefore, it could be suggested that continuous intake of aspartame and consequential exposure to its metabolites affect human oocyte quality and embryo development. On the other hand, elevated glucose concentrations might explain our findings that sugar consumption is also associated with reduced oocyte quality.

The potential effects of female nutrition on the outcomes of ICSI have been investigated in several studies. Ferreira et al. (2010) demonstrated that female patients who consumed soft drinks had a higher probability of obesity, which in turn negatively influenced the fertilization rate and the chance of miscarriage. In fact, gene activation can be changed by nutritional factors (Englund-Ogge et al., 2012), and so the effects of diet may be passed on to the progeny and influence pregnancy outcome (Cutfield et al., 2007) and the risk of future diseases (Barker, 2007; Barker et al., 2006). In addition, it has been demonstrated that being on a weight-loss diet had a negative influence on the likelihood of blastocyst formation, implantation rate and pregnancy chance (Braga et al., 2015). This is important because artificial sweetener consumption, specifically consumption of diet beverages, increased the most among females (Sylvetsky et al., 2012). This may be explained by the fact that women tend to control their weight through reduced-calorie diets, and it has been demonstrated that people on a weight loss/maintenance diet are the heaviest consumers of artificially sweetened soft drinks (Phelan et al., 2009).

Please cite this article in press as: Amanda Souza Setti, Daniela Paes de Almeida Ferreira Braga, Gabriela Halpern, Rita de Cássia S. Figueira, Assumpto IaconelliJr, Edson BorgesJr, Is there an association between artificial sweetener consumption and in-vitro reproduction outcomes?, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.11.004

Our study has limitations. The major limitation is the fact that the exact amounts of sugar and artificial sweetener ingested were not considered. It is important to note that: (i) the assessment method was not validated; (ii) given the self-reported nature of the dietary data used, the possibility of misreporting cannot be ruled out; (iii) because we did not take into account the consumption of artificial sweeteners and sugar from sources other than soft drinks and coffee, our data may have underestimated true consumption levels; (iv) it is difficult to distinguish between the effects of the various artificial sweeteners consumed alone and their effects when combined; (v) the consumption of sugar and artificially sweetened beverages was reported only at the beginning of ICSI treatment, and subsequent consumption was not taken into account; (vi) it is not possible to define whether our findings are derived by the effects of these beverages or by other associated dietary or socio-economic factors; (vii) we did not measure serum or follicular fluid glucose levels in order to make accurate correlations with the investigated parameters.

Additionally, there are limitations intrinsic to the kind of questionnaire used. Frequency questionnaires use a closed-ended form, have low accuracy and require accurate evaluation (Shim et al., 2014). On the other hand, their strengths are their suitability for epidemiological studies, their cost-effectiveness and time savings, and the simple assessment of usual dietary intake (Shim et al., 2014).

In an elegant review of the literature, Olivier et al. (2015) pointed out that no data exist to justify the substitution of sugars by artificial sweeteners. The review also stated that the reduction of sugar intake levels should be obtained through a reduction in the intake of sweet foods, and that artificially sweetened and sugar-sweetened soft drinks should not be consumed instead of water.

To our knowledge, this is the first study investigating the potential adverse effects of sugar and artificial sweeteners on oocyte quality and ICSI outcomes. The general population believes that artificial sweeteners are healthier than regular sugar, and is not aware of the dangers hidden behind the promise of reduced-calorie food and beverages. Our study highlights the importance of nutritional counselling prior to the beginning of IVF treatments, especially regarding the adverse effect of sugar and mainly artificial sweeteners on the success of assisted reproduction treatments.

In conclusion, our findings suggest that women consuming ≥3 servings of regular soft drinks or any amount of diet soft drinks are at an increased risk of oocyte dysmorphisms. Unfavourable dose-dependent embryo development on days 2 and 3 was observed in women consuming artificially sweetened coffee or artificially sweetened soft drinks. Negative dose-dependent influences on blastocyst formation, implantation rate and pregnancy chance were observed in women consuming 2 and ≥3 servings per day of diet soft drinks. Patients should be advised about the adverse effects of consuming artificial sweeteners, regular soft drinks and, in particular, diet soft drinks on the success of ICSI treatment.

ARTICLE INFO

Article history:
Received 5 June 2017
Received in revised form 13 November 2017
Accepted 14 November 2017
Declaration: The authors report no financial or commercial conflicts of interest.

Keywords:
Eating habits
Female infertility
Intracytoplasmic sperm injection
Lifestyle
Sweeteners

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