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ARTICLE

The impact of food intake and social habits on embryo quality and the likelihood of blastocyst formation

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Abstract The aim of this study was to evaluate the influence of patients' lifestyle factors and eating habits on embryo development. A total of 2659 embryos recovered from 269 patients undergoing intracytoplasmic sperm injection cycles were included. The frequency of intake of food items and social habits were registered and its influences on embryo development evaluated. The consumption of cereals, vegetables and fruits positively influenced the embryo quality at the cleavage stage. The quality of the embryo at the cleavage stage was also negatively correlated with the consumption of alcoholic drinks and smoking habits. The consumption of fruits influenced the likelihood of blastocyst formation, which was also positively affected by the consumption of fish. Being on a weight-loss diet and consumption of red meat had a negative influence on the likelihood of blastocyst formation. The likelihood of blastocyst formation was also negatively influenced by the consumption of alcoholic drinks and by smoking habits. The consumption of red meat and body mass index had a negative effect on the implantation rate and the likelihood of pregnancy. In addition, being on a weight-loss diet had a negative influence on implantation rate. Our evidence suggests a possible relationship between environmental factors and ovary biology.

KEYWORDS: eating habits, female infertility, food intake, intracytoplasmic sperm injection, lifestyle

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Introduction

Infertility affects between 8 and 16% of reproductive-aged couples (Stephen and Chandra, 2006). Over the past 2 decades, the use of assisted reproduction techniques has increased dramatically worldwide, and has made pregnancy possible for many infertile couples. Although high-quality embryos may be available for transfer, however, most in-vitro-produced embryos fail to implant (de Mouzon et al., 2012).

The identification of factors that may influence the implantation of in-vitro-produced embryos is one of the most studied areas in assisted reproduction techniques. Special attention has been paid to the effects of the patient's lifestyle on the outcomes of assisted reproduction techniques. Lifestyle factors and nutritional status are known to be critical determinants of normal reproductive function (Chavarro et al., 2008). Menopause has been reported to occur earlier (Midgette and Baron, 1990) and conception to be delayed for over 1 year (Baird and Wilcox, 1985) among smokers compared with non-smokers. Moreover, cigarette smoking may impair sperm motility, decrease antioxidant activity in the seminal plasma (Pasqualotto et al., 2008) and lead to increased thickness of the zona pellucida (Shiloh et al., 2004).

Obesity and low body weight may also impair fertility. A high body mass index (BMI) has been shown to adversely affect the outcomes of assisted reproduction technique treatment (Fedorcsak et al., 2004; Nichols et al., 2003), and physical activity positively affects embryo implantation and the chance of pregnancy (Ferreira et al., 2010). Other aspects of the patient's diet may affect fertility, such as certain vitamins and food groups that could have a greater effect on reproductive health for both males and females (Chavarro et al., 2007, 2008; Keskes-Ammar et al., 2003; Mendiola et al., 2010; Silver et al., 2005).

Extended embryo culture and the transfer of blastocyst-stage embryos are associated with increased implantation rates compared with cleavage-stage embryo transfer (Blake et al., 2007; Papanikolaou et al., 2008). Prolonging the culture period allows for a better selection of embryos for transfer because laboratory assessment is undertaken after the embryonic genome has begun to be expressed (Tesarik et al., 1988). Moreover, because of their high implantation rate, single-blastocyst transfers may increase the pregnancy rate and reduce the frequency of multiple gestations (Gardner et al., 2004; Ryan et al., 2007). The proportion of embryos that develop to the blastocyst stage, however, is still variable (Mercader et al., 2003; Schoolcraft and Gardner, 2001; Westphal et al., 2003), and eventually, embryo transfer cancellation may occur in extended embryo culture programmes.

Different factors may be responsible for the variation in the rate of blastocyst formation, such as the culture media (Biggers and Racowsky, 2002; Macklon et al., 2002; Sepulveda et al., 2009), laboratory conditions (Lane et al., 2008; Meintjes et al., 2009; Waldenstrom et al., 2009), oocyte quality (Catala et al., 2012; Lin et al., 2003) or sperm origin (Nilsson et al., 2007). Less is known, however, about the influence of patients' lifestyle on embryo quality and blastocyst formation. Therefore, the goal of the present study was to evaluate whether patients' lifestyle factors and eating habits can influence embryo quality, the likelihood of blastocyst formation and clinical outcomes in patients undergoing assisted reproduction techniques.

Materials and methods

Study design

The study included 2659 embryos recovered from 269 patients undergoing intracytoplasmic sperm injection (ICSI) cycles between January 2012 and July 2013. All patients completed a questionnaire with multiple-choice questions before the beginning of the treatment. The women were asked about the frequency of their consumption of many food items and about their social habits.

The effects of dietary and social habits on embryo quality on day three and the likelihood of blastocyst formation were evaluated. To evaluate the effects of dietary and social habits on the likelihood of blastocyst formation, 1400 embryos cultured until day 5 of development were evaluated. Moreover, the influence of dietary and social habits on clinical pregnancy rates was also investigated.

Clinical pregnancy was defined as the presence of fetal heart activity by ultrasound at 6 to 7 weeks of gestation after embryo transfer. 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 on 19 December 2012.

Food consumption and social habits frequency questionnaire

All patients were interviewed face-to-face before the beginning of the treatment. The modified validated questionnaire (Ribeiro et al., 2006) contained multiple-choice questions about the average frequency of consumption of food items during the past year.

The food categories investigated in the present study were cereals, vegetables, legumes, fruits, red and pork meat, chicken, fish, dairy products, chocolate, soft drinks, caffeine-containing soft drinks, alcoholic drinks, dietary sweetener and coffee.

The frequency of food consumption was registered on a scale with five values, ranging from no consumption to repeated daily consumption. The specific categories were never or less than once per month; one to three times per month; once per week; two to four times per week; and every day.

The questionnaires were completed by the same interviewer, and a different questionnaire recorded information on exercise, weight-loss dieting in the past 3 months, the number of meals eaten per day and smoking habits. In addition, BMI was measured.

Exercising was recorded on the following scale: less than 1 h per week; 1 h per week; 2 h per week; 3 h per week; 4 h per week; and 5 h or more per week. Smoking habits were recorded as the number of cigarettes smoked per day, and BMI was measured based on weight/height^2 and expressed as Kg/m^2.

Ovarian stimulation

Ovarian stimulation was achieved by pituitary suppression using a gonadotrophin-releasing hormone antagonist (Cetrotide; Serono, Geneva, Switzerland), and the ovarian stimulation was performed using recombinant FSH (Gonal-F; Serono, Geneva, Switzerland).

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 ultrasound ovum retrieval.

Preparation of oocytes

The retrieved oocytes were maintained in culture media (Global® for fertilization; LifeGlobal, CT, USA) supplemented with 10% protein supplement (LGPS; LifeGlobal, CT, USA) and covered with paraffin oil (Paraffin oil P.G.; LifeGlobal, CT, USA) 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, CT, USA). The remaining cumulus cells were mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, USA).

Oocytes that were observed to have released the first polar body were considered mature and were used for ICSI, which is carried out for all IVF cycles in this centre.

Intracytoplasmic sperm injection

A micro-injedtion dish prepared with 4 μl droplets of buffered medium (Global® with HEPES; LifeGlobal, CT, USA) was used to carry out ICSI and covered with paraffin oil on a heated stage (37.0 ± 0.5°C) of an inverted microscope. About 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body. The embryos were maintained in a 50 μl drop of culture medium (Global®; LifeGlobal, CT, USA) supplemented with 10% protein supplement and covered with paraffin oil in a humidified atmosphere under 6% CO_2 at 37°C for 3 days.

Embryo morphology evaluation

Embryo morphology was assessed as follows: 16–18 h after ICSI ± 1 h; 44 h ± 1 h after ICSI, for day two evaluation; 68 h ± 1 h after ICSI, for day 3 evaluation; 116 h ± 2 h after ICSI for day 5 evaluation; and 120 h ± 2 h after ICSI for late day 5 evaluation (transfer time). Embryo morphology was carried out using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon, Tokyo, Japan) with a Hoffmann modulation contrast system under ×400 magnification.

To evaluate cleavage-stage morphology, the following parameters were recorded: the number of blastomeres; the percentage of fragmentation; the variation in blastomere symmetry; the presence of multinucleation; and the defects in the zona pellucida and cytoplasm. High-quality cleavage-stage embryos were defined as those with all of the following characteristics: eight to 10 cells on day 3; less than 15% fragmentation; symmetric blastomeres; the absence of multinucleation; colourless cytoplasm with moderate granulation and no inclusions; the absence of perivitelline space granularity; and the absence of zona pellucida dysmorphism. Embryos lacking any of these characteristics were considered to be of low quality.

To evaluate blastocyst formation, the embryos were classified based on their degree of expansion and hatching status, as follows: (i) an early blastocyst with a blastocoel that was less than one-half of the volume of the embryo; (ii) a blastocyst with a blastocoel that was greater than one-half of the volume of the embryo; (iii) a full blastocyst with a blastocoel that completely filled the embryo; (iv) an expanded blastocyst; (v) a hatching blastocyst; and (vi) a hatched blastocyst. Full, expanded, hatching and hatched blastocysts were considered for the blastocyst group, and the other embryos were considered for the non-blastocyst group.

Statistical analysis

Regression analyses were conducted to study the influence of specific food and beverage intake on the cleavage-stage embryo quality, blastocyst formation, implantation and the likelihood of pregnancy. Binary regression analyses were used when consumption was analysed (dichotomous variable), and ordinal regression analyses were used when the frequency of consumption (ordinal variable) was analysed. All regression analyses were adjusted for maternal age, the number of retrieved oocytes and the fertilization rate, as these variables were considered to be potential confounders in the association between the factors evaluated and the outcomes. The results were expressed as odds ratios (OR), 95% confidence intervals (CI) and *P*-values. The results were considered to be significant at the 5% critical level ($P < 0.05$). The data analysis was conducted using Minitab Statistical Software (version 16).

Results

Influence of food intake and social habits on embryo quality and the likelihood of blastocyst formation

The consumption of cereals (OR 1.34, CI 1.09 to 1.59; $P = 0.016$), vegetables (OR 1.25, CI 1.06 to 1.38; $P = 0.025$) and fruits (OR 1.38, CI 1.07 to 1. 71; $P = 0.018$) positively influenced the embryo quality at the cleavage stage. The quality of the embryo on day 3 was negatively correlated with the consumption of alcoholic drinks (OR 0.75, CI 0.62 to 0.95; $P = 0.032$) and with smoking habits (OR 0.95, CI 0.82 to 0.99; $P = 0.044$) (Table 1).

The quality of embryo on cleavage stage and also the conversion of embryos into blastocyst was influence by some food intake and other habits. An increase chance of formation of

Table 1 Regression analysis of eating and social habits that may affect the cleavage-stage embryo quality.

Response variable	*Predictor cariable*	*OR*	*CI lower*	*CI upper*	*P-value*
Cleavage-stage embryo quality	Cereals	1.34	1.09	1.59	0.016
	Vegetables	1.25	1.06	1.38	0.025
	Legumes	1.02	0.86	1.32	NS
	Fruits	1.38	1.07	1.71	0.018
	Red meat	0.81	0.65	1.00	NS
	Fish	1.05	1.01	1.36	NS
	Chicken	1.00	0.80	1.26	NS
	Dairy products	1.01	0.80	1.27	NS
	Chocolate	1.16	0.94	1.43	NS
	Alcoholic drinks	0.75	0.62	0.95	0.032
	Soft drinks	0.92	0.82	0.104	NS
	Caffeine-containing soft drinks	1.13	0.77	1.68	NS
	Dietary sweetener	0.89	0.76	1.25	NS
	Coffee	1.33	0.99	1.63	NS
	Exercise	1.07	0.84	1.36	NS
	Weight-loss diet	0.89	0.68	1.17	NS
	Smoking	0.95	0.82	0.99	0.044
	Meals per day	0.98	0.97	1.02	NS
	BMI	0.99	0.96	1.01	NS

BMI = body mass index; CI = confidence interval; NS = not statistically significant; OR = odds ratio.

Table 2 Regression analysis of eating and social habits that may affect the likelihood of blastocyst formation.

Response variable	*Predictor cariable*	*OR*	*CI lower*	*CI upper*	*P-value*
Likelihood of blastocyst formation	Cereals	1.01	0.80	1.28	NS
	Vegetables	1.02	0.90	1.02	NS
	Legumes	1.88	0.88	1.33	NS
	Fruits	1.32	1.08	1.63	0.008
	Red meat	0.81	0.65	0.99	0.049
	Fish	1.31	1.05	1.66	0.018
	Chicken	1.00	0.80	1.26	NS
	Dairy products	1.01	0.80	1.27	NS
	Chocolate	0.89	0.68	1.17	NS
	Alcoholic drinks	0.75	0.62	0.95	0.032
	Soft drinks	0.92	0.82	0.104	NS
	Caffeine-containing soft drinks	1.13	0.77	1.68	NS
	Coffee	1.33	0.99	1.63	NS
	Dietary sweetener	1.25	0.98	1.55	NS
	Exercise	1.07	0.84	1.36	NS
	Weight-loss diet	0.78	0.62	0.98	0.046
	Smoking	0.76	0.56	0.94	0.045
	Meals per day	0.98	0.97	1.02	NS
	BMI	0.99	0.96	1.01	NS

BMI = body mass index; CI = confidence interval; NS = not statistically significant; OR = odds ratio.

blastocysts with a blastocoel that completely fills the embryo, expanded blastocysts, and a hatching or hatched blastocysts was observed among patients who consumed fruits (OR 1.32, CI 1.08 to 1.63; $P = 0.008$) or fish (OR 1.31, CI 1.05 to 1.66; $P = 0.018$). Being on a weight-loss diet (OR 0.78, CI 0.62 to 0.98; $P = 0.046$) and the consumption of red meat (OR 0.81, CI 0.65 to 0.99; $P = 0.049$) had a negative influence on the likelihood of blastocyst formation, and, in similarity to the cleavage-stage embryo quality, the likelihood of blastocyst formation was negatively influenced by the consumption of alcoholic drinks (OR 0.75, CI 0.62 to 0.95; $P = 0.032$) and by smoking habits (OR 0.76, CI 0.56 to 0.94; $P = 0.045$) (**Table 2**).

Although the blastocyst formation and the hatching status were correlated with food intake and social habits, no association with the blastocyst quality (inner cell mass and trophectoderm morphology) was observed.

Table 3 Binary regression analysis of eating and social habits that may affect the likelihood of clinical pregnancy.

Response variable	*Predictor cariable*	*OR*	*CI lower*	*CI upper*	*P-value*
Clinical pregnancy	Cereals	1.59	0.73	2.48	NS
	Vegetables	1.67	0.87	4.32	NS
	Legumes	1.93	0.33	2.47	NS
	Fruits	0.62	0.28	1.35	NS
	Red meat	0.68	0.48	0.89	0.042
	Fish	0.81	0.36	1.81	NS
	Chicken	0.98	0.66	1.02	NS
	Dairy products	0.71	0.33	1.55	NS
	Chocolate	1.06	0.63	1.77	NS
	Alcoholic drinks	1.02	0.69	1.50	NS
	Soft drinks	0.93	0.62	1.39	NS
	Coffee	0.83	0.54	1.26	NS
	Caffeine-containing soft drinks	1.23	0.88	1.45	NS
	Dietary sweetener	0.81	0.56	1.56	NS
	Exercise	1.69	0.86	2.70	NS
	Weight-loss diet	0.79	0.56	0.97	0.011
	Smoking	0.86	0.38	1.93	NS
	BMI	0.43	0.25	0.93	0.046

BMI = Body mass index; CI = Confidence interval; OR = odds ratio.

Influence of food intake and social habits on ICSI outcomes

The consumption of red meat (OR 0.68, CI 0.48 to 0.89; $P = 0.042$), BMI (OR 0.43, CI 0.25 to 0.93; $P = 0.046$) and being on a weight-loss diet (OR 0.79, CI 0.56 to 0.97; $P = 0.016$) had a negative effect on the likelihood of clinical pregnancy (**Table 3**).

Discussion

Recent studies have shown that infertility has drastically increased over the past decades (Bushnik et al., 2012). This can be explained by a number of reasons. In addition to the delay in childbearing observed with women being older when first attempting pregnancy, environmental factors have identified as affecting general health and the capacity to reproduce.

In the present study, the effect of specific food and beverage intake and other social habits on embryo quality, the likelihood of blastocyst formation and ICSI outcomes was evaluated. The results showed that the quality of the embryo and the likelihood of blastocyst formation were negatively influenced by the consumption of alcoholic drinks and by smoking habits.

Although it is well documented that cigarette smoke contains many harmful substances, more research is needed to establish a link to infertility (Roth and Taylor, 2001). Strong evidence supports the adverse effects of smoking on fertility, acting through different pathways (Augood et al., 1998; Sharara et al., 1994; Soares et al., 2007). Feichtinger et al. (1997) suggested that smokers require more assisted reproduction technique cycles than non-smokers do to achieve a pregnancy. Together with our findings, this evidence suggests that the substances that are inhaled through cigarette smoking may affect a growing follicle during gametogenesis, affecting the quality of the oocyte and thus the quality of the embryo, its developmental competence and its implantation potential.

In the present study, alcohol consumption in addition to cigarette smoking was shown to affect embryo development. Alcohol consumption has been reported to impair fertility, although the level of consumption associated with the risk is unclear. A high level of alcohol consumption is known to affect the fetus (Goransson et al., 2003; Krulewitch, 2005), but the effect on fertility at lower levels is still unknown. The mechanisms by which alcohol could impair conception are unclear but may include an alcohol-induced rise in oestrogen, which reduces FSH secretion, suppressing folliculogenesis (Homan et al., 2007).

In the present study, the questionnaire was conducted before starting treatment. Even though the nutritionist counsels the patients to stop or at least reduce smoking and alcohol habits before and especially during the treatment, we were, unfortunately, unable to ensure that this happened. In fact, many times we did not know if patients were smoking at the time of treatment, and this is a drawback of these types of studies.

Recently, the effect of pollutants and occupational factors on reproductive health has been a matter of debate. Environmental pollutants, such as methyl mercury, pesticides, lead, welding, organic solvents, radiation, xenoestrogens and household glues have been shown to have a negative effect on fertility (Bretveld et al., 2008; Foster et al., 2008; Homan et al., 2007; McDiarmid et al., 2008; Mendiola et al., 2008; Mendola et al., 2008). In the present study, the consumption of red meat had a negative effect on embryo development and the likelihood of pregnancy. This finding is consistent

with the poor fertility associated with a higher intake of products that may contain xenoestrogens, and endocrine-disrupting compound (EDC). The use of these EDC compounds in the food industry may result in an increased level of sex steroids in processed foods, such as meat or milk (Bushnik et al., 2012; Fusani et al., 2007; Milnes et al., 2006).

In addition, the negative effect of the consumption of red meat on embryo development and the likelihood of pregnancy may be due to the increase in the absorption of advanced glycation end-products (AGE). These are compounds created through the non-enzymatic reaction between reducing sugars and free amino groups in proteins, lipids or nucleic acids (Singh et al., 2001). Animal-derived foods that are high in fat and protein are generally rich in AGE and are also prone to new AGE formation during the cooking process (Uribarri et al., 2010). Formation and accumulation of AGE occurs during normal ageing and may cause tissue and intracellular damage directly by macromolecular trapping and cross-linking and also indirectly by binding to specific AGE receptors (RAGE) on cell surfaces (Thomas et al., 2005). Jinno et al. (2011) suggested that AGE accumulation was highly correlated with poor follicular and embryonic development and with a lower likelihood of ongoing pregnancy.

In a recent study by Mendiola et al. (2009), an association between semen quality and the consumption of foods containing processed meat (sausages and others) was observed, although the effect of these compounds on female fertility is less certain. It could be argued that partners usually share the same habits, so if one eats a certain group of foods, it is expected that the other may consume it as well. Therefore, the decreased embryo developmental competence and likelihood of pregnancy observed in these couples may be due to diminished sperm, rather than oocyte, quality.

Other food items were associated with increased embryo quality and developmental potential. Intake of cereals, vegetables and fruits led to better embryo development. Although a strong correlation between maternal eating habits and pregnancy outcomes has been demonstrated (Hambidge et al., 2014; Ota et al., 2012; Sagedal et al., 2013), the effect of female habits on conception success is still to be elucidated. Strong evidence shows, however, that a well-balanced, healthy diet is beneficial for general health (Sanders, 2004). Diet before pregnancy may also influence fetal well-being (Imdad and Bhutta, 2011; Imdad et al., 2011). In line with these findings, it is expected that reproductive performance may be positively influenced by the consumption of healthy food.

In line with this, previous studies have reported that fruit and vegetable intake is associated with lower oxidative stress and improved antioxidant status (Rink et al., 2013). Oxidative stress has been shown to cause defective embryo development *in vitro* (Agarwal et al., 2003). Bedaiwy et al. (2012) reported that higher follicular fluid total antioxidant capacity and reactive oxygen species (ROS) levels are associated with pregnancy after ICSI. Endogenous ROS have been shown to play important roles as signalling molecules, during reproductive events. It has also been suggested that ROS are key signals in the initiation of apoptosis in antral follicles and granulosa cells of antral follicles. Additionally, high concentrations of antioxidants in oocytes are necessary for normal fertilization and subsequent preimplantation embryonic development (for review see Devine et al., 2012).

Evidence suggests that couples undergoing infertility treatment are at increased risk for adverse reproductive effects from the widely distributed toxic elements such as mercury, lead, cadmium and arsenic (Kim et al., 2010). It has been reported that fish can be an exposure route for toxic chemicals, which confer risks for health outcomes (Kim et al., 2010; Mahaffey et al., 2009). The relationship between the risks and benefits of fish consumption, however, is complex. Consumption of fish and fish oils may be beneficial for early cognitive development and improve cardiovascular health in adults. A recent report demonstrated a positive effect of the consumption of fish oil on sperm parameters Alizadeh et al. (2014), in another study, showed that women with a high alpha-linolenic acid intake had a higher baseline oestradiol, and alpha-linolenic acid and docosahexaenoic acid intake may improve embryo morphology (Hammiche et al., 2011). This is in accordance with our findings, which demonstrated a positive relationship between the consumption of fish and the likelihood of blastocyst formation.

Our results also demonstrated that the likelihood of blastocyst formation and the pregnancy rate were negatively affected in patients who reported being on a weight-loss diet. It is well known that the reproductive system is extremely sensitive to influences from the external environment and that the mechanisms responsible for the adjustment of reproductive function involve the availability of calories (Martin et al., 2004; Wade and Jones, 2004). These days, some people go through 'miraculous-sounding diets that claim to melt off pounds within few days'. It is known, however, that eating disorders leading to weight loss are associated with a reduced frequency or the cessation of ovulation. Food is used as a source of energy for a variety of essential and non-essential functions. In times of deprivation, it is necessary to ration available oxidizable substrates in favour of essential functions that are required to sustain life. Reproduction is expendable at least in the short term and can be deferred until times are more favourable. Women with anovulation associated with excessive physical activity or who are underweight have low levels of leptin, LH and oestradiol. The frequency of gonadotrophin pulses is too low to sustain the development of antral follicles to the point of ovulation (Sommer et al., 2006). In some female athletes when energy expenditure is reduced, such as after an injury, menstrual cycles return (Loucks, 2003).

Additionally, obesity is an important cause of subfertility in many modern societies (ESRHE, 2006). In the present study, BMI was shown to be inversely correlated with treatment success. This finding is in accordance with a previous report by our group, revealing that a high BMI may adversely affect the outcomes of assisted reproduction technique-based treatment (Fedorcsak et al., 2004).

Why the blastocyst formation chance was affected by some habits, but no correlation with the blastocyst quality was observed, is a matter of the debate. The most important of the reported advantages of blastocyst-stage transfer in IVF is that it yields a higher pregnancy rate than cleavage-stage embryo transfer. Previous studies have reported the importance of trophectoderm, but not inner cell mass morphology for blastocyst implantation (Ahlstrom et al., 2011; Hill et al., 2013). More recently, however, it was shown that the blastocyst formation (expansion and hatching status) is the most important parameter affecting blastocyst implantation (Van den

Abbeel et al., 2013). Therefore, it would be expected that specific food intake and social habits, which affects the cycle's outcomes, would preferably have an effect on the blastocyst formation expansion and hatching status rather than on the trophectoderm and inner cell mass morphology. There is, however, a need for increased knowledge of the effect of each specific morphology parameter at the blastocyst stage on the probability of successful implantation and pregnancy.

One limitation of the present study is the lack of information concerning food intake and social habits in men, which would bias the results. We believe, however, that the oocyte quality plays a vital role in the blastocyst formation and embryo implantation; moreover, as discussed previously, it could be argued that partners usually share the same habits, so if one eats a certain group of foods, it is expected that the other may consume it as well.

In summary, our findings have shown that embryo developmental potential and clinical outcomes may be influenced by food intake and social habits. These results suggest that couples seeking assisted reproduction techniques must be advised about the adverse effects of women's lifestyle on treatment success and highlight the importance of involving a nutritionist in assisted reproduction technique programmes.

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