Nutrition, lifestyle and the implantation potential of a pre-implantation embryo.

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Samenvatting

Introductie: Subfertiliteit is een toenemend probleem in Nederland. Vruchtbaarheids-behandelingen worden toegepast om koppels te helpen met zwanger raken. De Embryoscoop is een nieuwe techniek die wordt gebruikt bij vruchtbaarheidsbehandelingen, om embryo's te cultiveren in optimale omstandigheden. De Embryoscoop maakt iedere 20 minuten een foto, deze beelden maken het volgen van de embryonale ontwikkeling mogelijk. De beelden worden gebruikt om de beste embryo's te selecteren en om het KIDScore algoritme te ontwerpen; een score die het implantatiepotentieel van het embryo voorspelt. De periconceptionele periode is belangrijk omdat juist in deze periode negatieve zwangerschapsuitkomsten ontstaan. Ongezonde eet- en leefgewoonten in deze periode kunnen een negatieve invloed hebben op de vruchtbaarheid en de ontwikkeling van een embryo, ook later in het leven. SlimmerZwanger is een online applicatie, die ondersteuning biedt aan koppels die zwanger willen worden. De applicatie heeft als doel zwangerschaps-kansen vergroten en bijdragen aan gezonde embryonale en feutale ontwikkeling door het verbeteren van eet- en leefgewoonten.
Doel: Het doel van dit onderzoek is het bestuderen van de relatie tussen de 5 kenmerken van SlimmerZwanger (inname van groente, inname van fruit, foliumzuur suppletie, roken en alcohol gebruik) en het implantatiepotentieel van een pre-implantatie embryo, gecultiveerd in de Embryoscoop, gedefinieerd als KIDScore.
Methode: De onderzoekspopulatie van deze cross-sectionele studie bestaat uit 79 deelnemers (N=79), die vruchtbaarheidsbehandelingen kregen in het Erasmus Medisch Centrum en deelnamen aan SlimmerZwanger tussen oktober 2014 en november 2016. De embryo's van de deelnemers werden in de Embryoscoop gecultiveerd. Gegevens geregistreerd door de Embryoscoop zijn gebruikt om de KIDScore te berekenen. Gegevens van de uitgangsmeting van SlimmerZwanger zijn gebruikt om de 5 kenmerken van eet- en leefgewoonten te definiëren. Deze voeding en leefstijlgegevens zijn vertaald in risicoscores. Na een willekeurige selectie van 1 embryo per vrouw, is logistische regressie toegepast om de associatie tussen voeding, leefstijl en het implantatiepotentieel van een embryo te analyseren.
Resultaten: Uit de resultaten blijkt een significante negatieve relatie tussen de voedingsrisico score (VRS) en de KIDScore (OR 0.76, CI 0.58-0.98, p-waarde 0.04). Leefstijlfactoren (onvoldoende foliumzuur suppletie, roken en alcohol gebruik) kwamen onvoldoende voor in de metingen voor verdere analyse.
Conclusie en discussie: De vastgestelde relatie geeft aan dat vrouwen met een hogere VRS en lagere kans hebben op het ontwikkelen van een embryo met een hoog implantatie potentieel. De associatie tussen voeding en embryonale ontwikkeling wordt bevestigd door recent onderzoek. Ongezonde leefstijl wordt negatief geassocieerd met embryonale ontwikkeling. Beperkend voor dit onderzoek is dat de subfertiele populatie, met voornamelijk mannelijke oorzaken voor onvruchtbaarheid, niet representatief is voor de algehele populatie. De KIDScore kan niet toegekend worden aan alle embryo's en de gebruikte risicoscore is nog niet gevalideerd. Sterke punten van dit onderzoek zijn innovatieve onderzoeksdata en de unieke onderzoekspopulatie. Toekomstig onderzoek moet meer inzicht verschaffen over factoren die effect hebben op embryonale ontwikkeling en gedragsverandering.
Aanbevelingen: Stellen die vruchtbaarheidsbehandelingen krijgen, moeten voeding en leefstijl advies ontvangen. Preconceptie zorg verdient meer aandacht en moet toegankelijk zijn voor alle koppels met een kinderwens.

Abstract

Introduction: Subfertility is a growing problem in The Netherlands. Assisted reproductive techniques (ART) are applied to aid subfertile couples conceiving. The EmbryoScope is a novel incubator, used in ART, to cultivate embryos under optimal conditions. The EmbryoScope provides time lapse images, which enable close monitoring of early embryo development. Time lapse images generated by the EmbryoScope are used to select the embryos with the highest implantation potential and to design the KIDscore; a score predicting embryonic implantation potential. Embryo development during the periconceptional period is vital because in this period most reproductive failures originate. In this period, poor nutrition and lifestyle can have a negative impact on the development and health of an embryo, both immediate and later in life. SmarterPregnancy is an online application, supporting couples trying to conceive. SmarterPregnancy aims to improve chances of conceiving and contribute to healthy development of the embryo by modifying nutrition and lifestyle behavior.
Aim: The aim of the present study is to examine the relation between the five SmarterPregnancy characteristics (intake of fruits, intake of vegetables, folic acid supplementation, smoking and use of alcohol) and the implantation potential of a pre-implantation embryo cultured in the EmbryoScope, defined by the KIDscore.
Methods: The study population of this cross-sectional study consists of 79 participants that underwent ART treatment in the Erasmus Medical Center and subscribed to SmarterPregnancy between October 2014 and November 2016. Participant's embryos were placed in the EmbryoScope for cultivation. Data registered by the EmbryoScope was used to calculate the KIDScore. Baseline data from SmarterPregnancy were used to define the 5 nutrition and lifestyle characteristics. This data was translated in risk scores. After randomly selecting one embryo per participant, logistic regression was conducted to analyze the association between nutrition, lifestyle and the implantation potential.
Results: Results show a significant negative relation between the nutrition risk score (NRS) and the KIDScore (OR 0.76, CI 0.58-0.98, p-value 0.04). Lifestyle behaviors (inadequate intake of folic acid, smoking and the use of alcohol) were insufficient present in the measurements to be analyzed.
Conclusion and discussion: The relation found in this study indicates that women with a higher NRS have a lower chance to develop a high-quality pre-implantation embryo. The association between nutrition and embryo development is supported by recent research. Detrimental lifestyle behavior showed to be negatively associated with embryo development. Limitations are that the present subfertile population, with predominant male subfertility causes, is not representative for the general population. The KIDScore was not assigned to all embryos and the risk scores used are not yet validated. Strengths are the high quality innovative data used and the unique research population. Future research should aim at more knowledge about factors impacting embryo development and behavioral change.
Recommendations: Couples undergoing ART should receive nutrition and lifestyle advice. Preconception care should receive more attention and made easily available for all couples with a desire to have a child.

Table of contents

List of abbreviations

2PN	Two pronuclei
ART	Assisted reproductive technique
AUC	Area under the curve
BMI	Body mass index
CE-1	Conformité Européenne, classe 1
CI	Confidence interval
HBO	Dutch vocational education; level high
HEAO	Advanced commercial education
HTS	Dutch technical education; level high
ICSI	Intra-cytoplasmatic sperm injection
IVF	In vitro fertilization
KID	Known implantation data
LBO	Dutch vocational education; level low
LEAO	Junior secondary commercial education
LHNO	Dutch vocational education; level low
LTS	Dutch technical education; level low
MBO	Dutch vocational education; level intermediate
MEAO	Secondary commercial education
MTS	Dutch technical education; level intermediate
NCD	Non-communicable diseases
NRS	Nutritional risk score
OR	Odds ratio
PABO	Teachers college for primary education
PH	Potential of Hydrogen
P-value	Probability value
SD	Standard deviation
SMS	Short message service
tPNF	Time to pronuclear fade
TRS	Total risk score
VBO	Dutch preparing vocational education; level low
VCM	Volume x Concentration of sperm cells x Motile spermatozoa percentage
VRS	Voedings risicoscore

Introduction

1.1 Subfertility

Subfertility (no conception < 12 months) is a growing problem in the Western world, highly impacting people's lives. One in six couples in The Netherlands are considered subfertile and 5% of all couples remain involuntary childless (1, 2). In 2015, in The Netherlands, one in every 36 children were born using the assisted reproductive technique (ART) in vitro fertilization (IVF) (3). IVF is a commonly used ART, whereby - after ovarian stimulation treatment using hormones to stimulate the growth of oocytes - oocytes are retrieved and fertilized outside the female body and cultivated in a laboratory. Another form of ART is intra-cytoplasmatic sperm injection (ICSI), where a single sperm cell (or sperm head or nucleus) is injected into the oocyte. IVF treatment requires a large number of sperm cells to fertilize the oocyte, whereas ICSI is applied to cases with low VCM (VCM is a measure for semen that consists of: Volume x Concentration of sperm cells x Motile progressive spermatozoa percentage) (4). After fertilization, between day 3 and day 5 after follicle punction, the embryo is transferred back in to the uterus (5). In The Netherlands, in 2015, 32,6% of the IVF/ICSI cycles resulted in an ongoing pregnancy (3). Couples undergoing IVF/ICSI are unique for research purposes, because fertilization and early development of the embryo takes place outside the uterus, in a laboratory, where development of the pre-implantation embryo can be monitored and studied.

In the laboratory, cultivation and selection of the most viable embryos for transfer is paramount to the success of IVF (6). The main dilemma in IVF is balancing between optimal cultivation and optimal selection. Ideally, multiple sequential evaluations are necessary to gather enough information to select the embryo with the best chance of implantation, leading to ongoing pregnancy. However, in removing the embryo from the standard incubator for evaluation, the embryo is exposed to potentially embryo toxic conditions (change in temperature, O_2 levels, PH, light exposure) outside the incubator, detrimental to the development of the embryo (6-8). A recently introduced technique, the EmbryoScope, can partially resolve this dilemma.

1.2 EmbryoScope

The EmbryoScope is a novel incubator, which incorporates a specialized built-in microscope designed for automated time-lapse embryo assessment by acquiring images. The EmbryoScope provides a controlled culture environment and captures comprehensive information on embryo development without handling or disturbing the embryo (6, 7, 9). The EmbryoScope registers information referred to as morphokinetics. The standard incubator provided information about the form or shape ("morpho") of the embryo. In addition, the EmbryoScope provides time-lapse images, thereby adding timed information on movement or timing ("kinetics") of the embryonic development. The use of morphokinetics, or timing of embryonic developmental events and visualization of dynamic morphology, available through continuous time-lapse monitoring, has added another dimension to current traditional morphology classification scores, used to predict embryo implantation potential and viability (10, 11). Time-lapse monitoring has been available for clinical routine since 2008 and to the Erasmus Medical Centre (EMC) since 2012 (12).

1.3 Morphokinetic algorithms

Traditionally, the embryo was selected based on morphology only, such as the number of pronuclei, the cleavage rate and the amount of fragmentation (13). The new information captured by the EmbryoScope initiated a search for morphokinetic parameters indicating which embryos are most likely to implant and result in pregnancy. In 2010, Wong showed that success or failure in human embryo development is largely determined before day 3 (14). A day-3 embryo is also referred to as a pre-implantation embryo. Wong also discovered the first morphokinetic parameters predicting the progression from a fertilized oocyte, referred to as a zygote, until the development of the embryo on day 5, also known as a blastocyst (14). The discovery of morphokinetic parameters was followed by the development of new prediction models and algorithms, from now on referred to as algorithms. In 2011, Meseguer introduced the first algorithm, followed by Conahan (2013) launching the Eeva selection algorithm, followed by Campbell (2013), Vermilyea (2014), Basile (2015), Milewski (2016), Motato (2016) and Liu (2016) (7, 15-21). As more knowledge and data became available, predictive power of algorithms improved.

The algorithms have been tested in several studies. Between 2011 and 2014 strong indications were found demonstrating that morphokinetic data can improve embryo selection compared to the traditional selection methods (6, 8, 13, 16, 17, 22-27). However, many of these studies were retrospective single center studies with a relatively small sample included for analysis. In 2014, Kaser published a systematic review, concluding that time-lapse monitoring has the potential to revolutionize clinical embryology, however there is currently no high-quality data originating from robust prospective studies to support the clinical use of this technology for selection of human pre-implantation embryos (28). In 2015, Cochrane published a systematic review analyzing 3 RCT's, concluding more data is needed to favor EmbryoScope over the standard incubator (29). After publication of these systematic reviews several prospective studies were published. A RCT by Rubio in 2014 found an increase in continuing pregnancy rates using continuous time-lapse culture and selection compared to standard embryo culture. In a prospective study by Adamson (2016), implantation and pregnancy rates were significantly higher in the group where traditional evaluation of the embryos was combined with data from the EmbryoScope opposed to the control group of embryos, which were only traditionally evaluated (30). However, a RCT by Goodman in 2016 showed a trend in improvements in clinical reproductive outcome, but the results were not significant (10). A limiting factor in developing a general applicable algorithm so far has been the relative low numbers of cases included in these studies. Also, the majority of previous algorithms were based on data specifically targeted on a treatment or clinic or a chain of clinics. Different clinics use different culturing conditions which can affect the development of the embryo (12).

1.4 KIDScore

In 2016, a new algorithm for predicting implantation potential for pre-implantation embryos (AUC=0,650) was developed, called KIDScore (KID = Known Implantation Data), designed to be general applicable (12). General applicable entails that the algorithm is based on a large number of cases - 3275 from 24 different centers - and can be used for both IVF and ICSI treatments and in different culturing conditions. The KIDScore was validated using 11218 embryos from 31 different clinics. The KIDscore is a deselection algorithm and is therefore more general applicable for different centers and conditions opposed to a selection model, since time ranges representing optimum conditions vary between clinics (12).The KIDscore ensures applicability for both IVF and ICSI treatment by using time to pronuclear fade (tPNF) as a starting point for the algorithm, opposed to time of insemination, which is different for these treatments. The fading of the pronuclei is the moment the two pronuclei disappear, which is very clear to determine, and equal for both IVF and ICSI treatment. Figure 1 shows the early embryo development, in the third schematic cell the pronuclear fade is depicted. Normally a pre-implantation embryo has eight cells on day three, as shown in figure 1.

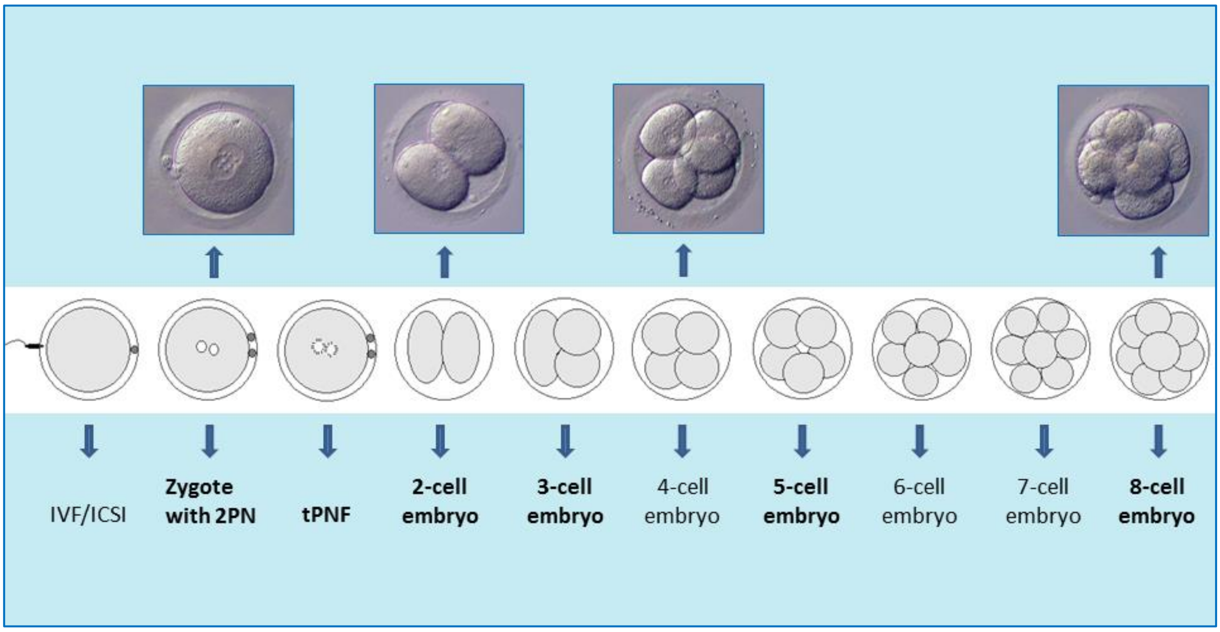

Figure 1. The development of an oocyte and sperm to pre-implantation embryo on day 3. 4 images captured by an EmbryoScope of human embryos are shown above the schematic representation of the embryos. The bold items are used in the KIDScore algorithm.

1.5 Embryo development and nutrition and lifestyle

Despite novel technical developments and the latest ART techniques, human implantation retains a high rate of early failure. Causes of embryonic arrest during pre-implantation development are poorly understood (27, 31). Recent studies point out that reproductive failures such as fertility problems, miscarriages, congenital malformations and fetal growth restriction largely originate in the periconceptional period (32-34). The periconceptional period (14 weeks before conception until ten weeks after conception) is crucial for understanding and improving fertility and health before, during and after pregnancy (33). In this period in particular, poor nutrition and lifestyle have negative impact on the development and health of an embryo, both immediate and later in life, thereby adversely affecting the health of future generations (32-37). Godfrey (2010), argues that in the case of non-communicable diseases (NCD's), which account for 60% of all deaths globally, interventions should take place early in life instead of later in life, when the disease manifests. Early is during and even before pregnancy. Altered maternal conditions can induce effects on the fetus that do not manifest until long after birth. For example, vascular structure in 9-year-old children is related to maternal diet before and during pregnancy. These effects, resulting from conditions inside the uterus, can change the DNA through methylations and thus carry on to the next generation (36).

Nutrition and lifestyle habits are modifiable factors that play an important role in reproductive health (2, 34, 38-42).The importance of healthy nutrition, healthy body mass index (BMI), use of folic acid and cessation of smoking and no use of alcohol in relation to reproductive health are supported by numerous studies (2, 34, 38, 39, 41-47). Couples contemplating pregnancy or early pregnant, as well as health care professionals, are often not aware of the detrimental effects of poor nutrition and lifestyle behaviors (39). In 2012, Twigt et al. investigated the association between adherence to general dietary recommendations in couples undergoing IVF/ICSI treatment and the chance of ongoing pregnancy. Improvement of nutrition of one point, expressed in the Preconception Dietary Risk score, resulted in an increase of ongoing pregnancy after IVF/ICSI treatment increased of 65% (40). These results led to the development of an online mobile health application, SmarterPregnancy, that supports users developing healthy behavior. The SmarterPregnancy coaching model is based on recent research on the influence of nutrition and lifestyle on fertility and pregnancy outcome, combined with behavioral theories on change and motivation. When compared to traditional health care, mobile health is efficient, cost effective and tailor made (41, 48). The online application SmarterPregnancy aims to improve nutrition and lifestyle behavior related to reproductive health in five areas proven to be effective in preconception care: intake of fruits, intake of vegetables, folic acid supplementation, smoking and use of alcohol (2, 34, 38, 39, 41-47).

Research on nutrition and lifestyle in the periconceptional period focusses on early embryonic development in relation to fertility and health of (future) parents and children. However, hardly any research is done on the relationship between the future parents' lifestyle and nutrition, in relation to the implantation potential of the embryo. Research on pre-implantation embryos has accelerated by the development of novel techniques like the EmbryoScope. Data generated by the EmbryoScope can be used to explore the possible connection between nutrition and lifestyle and early embryonic development. In 2015, Braga found a potential relation between nutrition and lifestyle habits and the quality of the pre-implantation embryo. Intake of fruits, vegetables and cereals had a positive relation with pre-implantation embryo quality and smoking and alcohol a negative relation (5).

In the present study, it was considered a logical next step to further investigate the potential relation between nutrition and lifestyle and pre-implantation embryonic development. The KIDscore will be used to define implantation potential of the embryos, together with data generated by the EmbryoScope and data collected through the SmarterPregnancy application.

1.6 Objectives and research question

The aim of the present study is to examine the relation between the five SmarterPregnancy characteristics (intake of fruits, intake of vegetables, folic acid supplementation, smoking and use of alcohol) and the implantation potential of a pre-implantation embryo cultured in the EmbryoScope, defined by the KIDscore.

This study will contribute to insight in healthy lifestyle and nutrition in relation to embryo development and implantation potential. Possible insight gained from this study may help develop knowledge supporting couples undergoing fertility treatment to improve chances of success. Eventually knowledge gained from research on couples undergoing ART could contribute to knowledge on nutrition and lifestyle in relation to embryonic health for the general population. Commissionaires goal is to gain knowledge on early embryo development in relation to nutrition and lifestyle, thereby aiming to improve success of IVF/ICSI treatment. Results of this study could be used in dietetic practice to advise couples suffering reproductive health problems to improve health conditions that may help improve the implantation potential of the pre-implantation embryo. By improving the health of the pre-implantation embryo, chances of successful reproduction may increase for couples in need of aid by ART. Knowledge gained could potentially lead to advice for couples with a desire to have children without the need for ART. In a broader view, this study contributes to research on preconception care. In 2007, a report was published advising the Dutch minister of Health to develop a program providing pre-conception care for every couple in The Netherlands with a desire for children (49). Hopefully this research can contribute to the understanding that nutrition and lifestyle are an important part of preconception care. This study may support dieticians to participate in providing preconception care.

Research question

What is the relation between the five SmarterPregnancy characteristics (intake of fruits, intake of vegetables, folic acid supplementation, smoking and use of alcohol) and the implantation potential of a pre-implantation embryo cultured in the EmbryoScope, defined by the KIDscore?

2. Methods

2.1 Study design and study population

In this cross-sectional study, baseline measurements derived from the SmarterPregnancy application were used. Couples who underwent IVF/ICSI treatment were recruited for the SmarterPregnancy program between October 2014 and November 2016. Data from these recruits of whom the embryos were cultivated in the EmbryoScope in the Erasmus MC in Rotterdam were included in this study. The data of the other participants was excluded. The study population consists of N=79 participants.

2.2 Ethical aspects

This study was conducted according to the principles of the Self-regulatory codes of conduct for Observational Research with personal data and the Erasmus MC research codes (50). This study analyzed data that were already available through routine IVF embryo culture and patients were not, and will not be, subjected to any procedures beyond routine IVF procedures. Digital informed consent was obtained for all participants, allowing the use of data for analysis (41). Data on embryo development was analyzed anonymously, with no attempts to trace back to the patients' identity (51).

2.3 Data collection embryos

The EmbryoScope Embryo Monitoring System (Unisense Fertilitech, Denmark) is an incubator designed for time-lapse embryo assessment, facilitated by a specialized built-in microscope. The EmbryoScope was tested and approved for use according the quality guidelines of the Dutch society for Embryology and the ISO norm 15189. During test research in 2011, the EmbryoScope allowed a significantly higher proportion of surplus embryos to develop to the expanded blastocyst stage (day 5 embryo), thereby proving to be safe for routine embryo culture (51, 52).

The images captured by the EmbryoScope were studied. When an event occurred the laboratory analyst made an annotation of the exact timing to match the event, and entered this in the EmbryoScope database. For each embryo, this data was stored into the EmbryoViewer database. All embryos annotated by the laboratory analysists for this study, were thereafter checked by the same embryologist, hereby minimalizing intra- and inter observer effects which contributes to the reliability of the measurements (53). Observations made using the time lapse images can be easily translated into quantitative data by using the entered timings. This data can be used to analyze developmental timings. Data on the cause of subfertility, type of treatment and number of oocytes retrieved are entered and saved in the EmbryoScope database.

To investigate the implantation potential of a pre-implantation embryo the KIDScore was used. The KIDScore ranges from 1-5 (ordinal variable) and is based on 6 annotations, 1 morphological annotation ensuring that the embryo had 2 pronuclei (2PN), and 5 timings.
1) 2 pronuclei (2PN)
2) time to pronuclear fade (tPNF)
3) time to 2-cell (t2)
4) time to 3-cell (t3)
5) time to 5-cell (t5)
6) time to 8-cell (t8)

These annotated timings were used to calculate a KIDScore for the embryos using the algorithm depicted in figure 2.

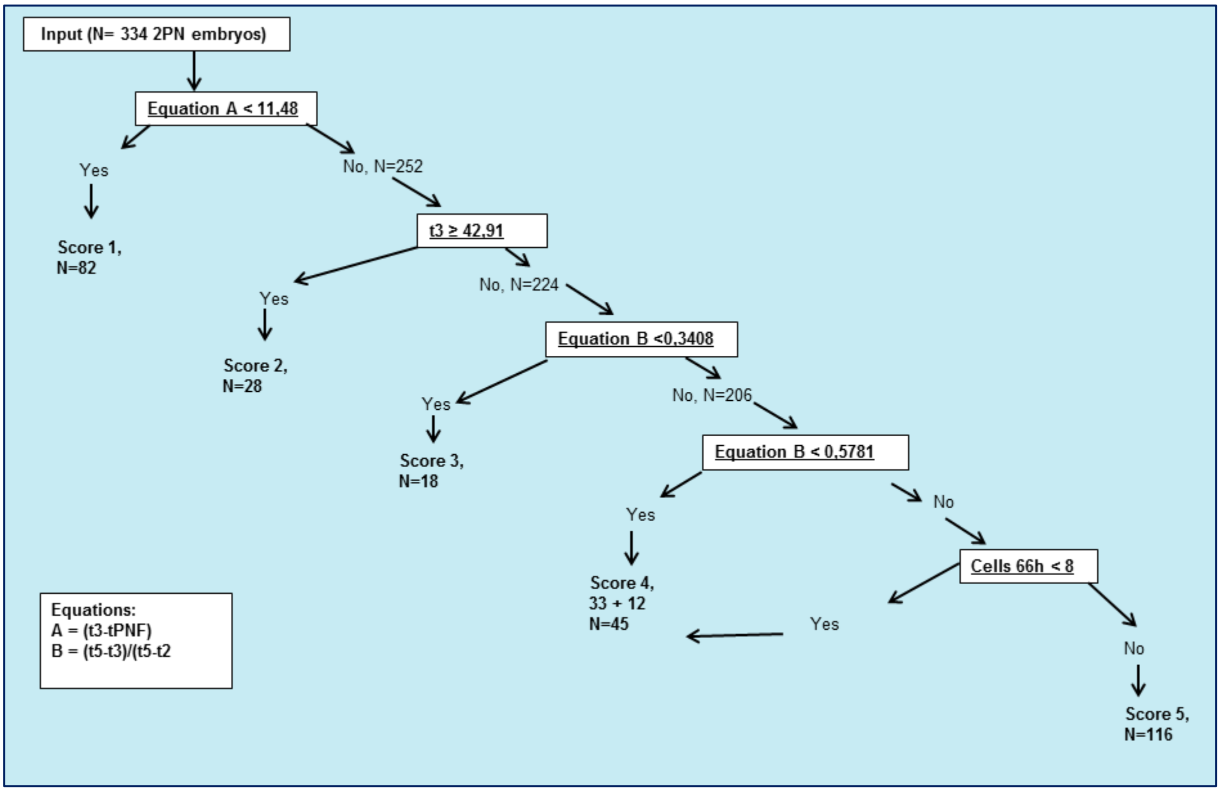

Figure 2: Classification Diagram of the KIDScore algorithm.

2.4 Data collection nutrition and lifestyle

The SmarterPregnancy program offers coaching during six months, focusing on the five most common detrimental nutrition and lifestyle behaviors with evidence based associations with pregnancy outcomes and fertility; inadequate intake of vegetables, fruit and folic acid and use of alcohol and smoking. Adequate intake was set at minimum of 200 grams of vegetables, two pieces of fruit and 400 micrograms of folic acid, no smoking and no use of alcohol. At baseline, a questionnaire was used to determine these factors. Vegetable intake was measured in grams, fruits in number of pieces, folic acid is determined by meeting the advised supplemental intake of 400 micrograms yes or no. Smoking and alcohol use was determined by marking as yes/no, and in case of a “yes” the quantity of smokes or drinks were noted. The questionnaires were designed by experienced researchers and healthcare professionals and were tested to be a reliable measurement instrument. SmarterPregnancy received the Conformité Européenne, classe 1 (CE-1), classifications (2013), and therefore complies with the highest rules of legislation for medical devices and is considered a reliable tool to improve the quality of medical care.

After subscription and completing the questionnaire, participants received individual coaching by SMS or email with a maximum of three messages per week during a six-month period. Every six weeks, the status on the five nutrition and lifestyle factors were reevaluated, using a questionnaire. In case of pregnancy the program adjusted to advise accordingly. Nutrition and lifestyle status were translated in a risk score for each behavior. A high-risk score represents unhealthier nutrition or lifestyle. Risks were depicted on a scale from 1-6. Vegetable and fruit intake were both subdivided into a risk score of 0, 1, 2 or 3, in which 0 represents an adequate daily intake (≥200 grams per day and ≥2 pieces per day, respectively). Score 1 and 2 both represent a ‘nearly adequate’ intake (vegetable intake of 150-200 gram and a fruit intake of 1.5–2 pieces per day), taken into account the presence (score 1) or absence (score 2) of the intention of the participant to change this risk factor.

Score 3 represents an inadequate daily intake (vegetable intake <150 gram and a fruit intake of <1.5 pieces). Folic acid supplement use was considered adequate (score 0) or inadequate (score 3) if a participant did or did not meet the recommendations of using a folic acid supplement of 400 µg daily during the periconceptional period. Risk scores with regard to smoking and alcohol consumption were based on the average daily use: no smoking (score 0), smoking 1-4 cigarettes (score 1), 6-14 (score 3) or ≥15 (score 6) and no drinking (score 0), drinking 1 (score 1), 1-2 (score 2) or ≥2 (score 3) alcoholic beverages. The total risk score (TRS) was defined as the sum of all risks per behavior. The nutrition risk score (NRS) score is the combined risk total of fruit, vegetable and folic acid intake. The design of the risk scores is based on extensive literature research and professional experience in the field of preconception care to ensure validity. The risk scores were used in research before (43).

2.5 Covariates

Various covariates were considered in this research, as they may be associated with the exposure variables (nutrition and lifestyle factors) and the outcome variable (KIDScore), namely BMI, age, ethnicity and education level. Regarding BMI, a high BMI is related to an unhealthy diet and lifestyle and has negative consequences for overall human health (54). Research shows that a high BMI (>25) leads to decreased fertility and decreases success rates in ART (55, 56). Embryos from overweight women display phenotypic and metabolic abnormalities and less embryos reach the blastocyst (day 5) stage opposed to women with a BMI < 25 (56, 57).

Age is a crucial factor in fertility and embryo development as the ovaries age faster than the rest of the female body, resulting in decline of both quality and quantity of oocytes. On average the decline starts at the age of 30 and at 45 most women cannot conceive any more (58-60). Ethnicity is related to fertility and embryonic development as research shows that ethnic background is associated with differences in ovarian reserve and fertilization, implantation and pregnancy rates (60-63). Ethnicity was categorized as either Western (Europe, North-America, Australia and New Zealand) or non-Western (other). Level of education is an important factor related to health, health behavior and fertility awareness (52, 60, 64). Level of education is categorized as low (VBO, LBO, LTS, LHNO, LEAO, MBO, MTS, MEAO etc.) and high (HBO, PABO, HTS, HEAO, university etc.) education. Data on ethnicity, age and level of education are derived from the SmarterPregnancy baseline measurements.

2.6 Statistical Analysis

The data collection was examined, assumption of normality for the continuous variables was verified with histograms and QQ plots.

Because the measurements collected on embryos are dependent per woman, randomization was applied to ensure validity of the results. One embryo per participant was randomly selected using a random function in SPSS. The selection criteria for the embryo was to have 2 pronuclei (2PN) as depicted in figure 1. These embryos were used to calculate the outcome variable, the KIDScore, using SPSS.

The outcome variable used in this study, the KIDScore, is distributed on an ordinal scale from one to five. This type of outcome distribution can be analyzed using ordinal logistic regression. However, in the original study the numbers of embryos were not evenly distributed between the groups (12). That, and the fact that the number of participants in this study are insufficient for ordinal logistic regression, led to the decision to divide the KIDScore into two groups. Embryos with a KIDScore of 1, 2, or 3 were labeled low implantation potential and embryos with a KIDScore of 4 or 5 were labeled high implantation potential. Logistic regression is a method of analysis used to analyze data with a binary outcome variable. Logistic regression analyses were conducted to analyze the association between the exposure variables, the TRS, NRS and intake of fruits and vegetables and the outcome

variable KIDScore. Fruits and vegetables were analyzed both as a continue variable and as a categorical variable, translated in adequate and inadequate intake.
The continue variables translated in a categorical variable to assess whether compliance to the recommendations was associated with implantation potential of the pre-implantation embryos.

The regression analyses were adjusted for potential confounders; maternal age, BMI, ethnicity and level of education. The covariates were added to the model when they changed the odds ratio (OR) > 10%. The results were expressed as odds ratio’s (OR), 95% confidence intervals (CI) and P-values. Results were considered statistically significant when the p-value was <0,05). SPSS version 21 was used to analyze the data.

3. Results

3.1 Study population

The study population consists of 79 participants (N=79). Randomization of one embryo per participant with the condition of two pronuclei was executed. Not all women produced an embryo, this randomized selection left 71 embryos for further analysis. For these 71 embryos, the KIDScore was calculated. Assigning a KIDScore to the embryos narrowed the final selection to 64 embryos. The fact that not every embryo could be assigned a KIDscore is consistent with the findings in the original study on the KIDScore algorithm (12). In figure 3 the flowchart of the study population is displayed.

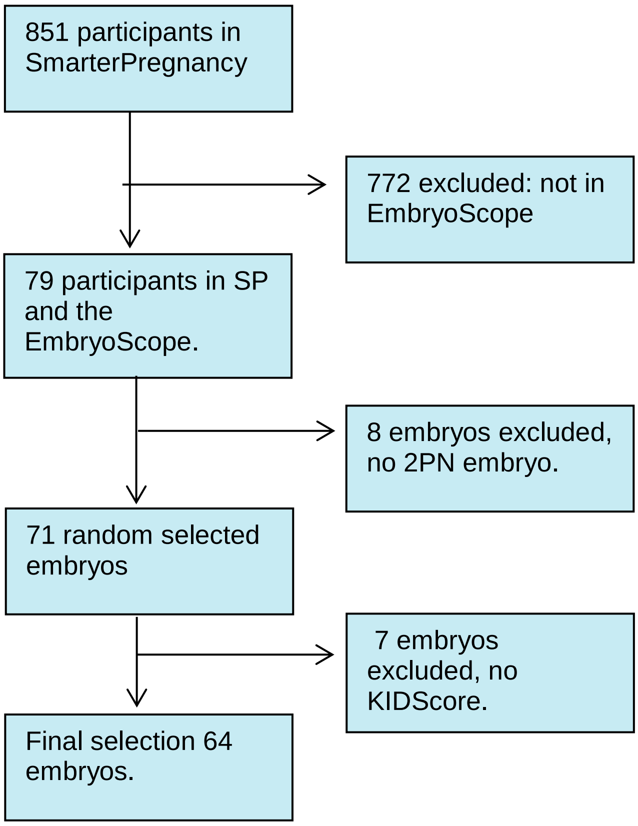

Figure 3: flowchart final study population. SP = SmarterPregnancy

The final selection of 64 participants is comparable to the original selection of 79 study participants. Table 1a, 1b and 1c depict the characteristics of the participants recruited for this study. The average age of the study population is 31,5 years, the average BMI is 24.4 kg/m^2. Of causes for subfertility, 90% of the cases in this dataset are male factors, explaining 98% ICSI treatments. In the Erasmus MC, the EmbryoScope is mainly used for ICSI treatments, this explains the high amount of ICSI treatments in this dataset. 31% of the participants have an adequate intake of vegetables and 52% of the participants have an adequate intake of fruits. In this study population, none of the participants' smokes, one participant has an inadequate use of folic acid supplementation and three participants used 1-7 alcoholic consumptions a week. These numbers of lifestyle factors are insufficient for logistic regression or any other statistical analyses.

Table 1a: General characteristics for the study population, depicted for the first selection (N=79) and after final selection (N=64).

POPULATION	**N=79**	**N=64**
Age mean, (SD)	32,1 (4,8)	31,5 (4,4)
BMI mean, (SD)	24,4 (3,9)	24,4 (4,0)
Ethnicity		
Western n, (%)	70 (89%)	57 (89%)
Non-Western n, (%)	9 (11%)	7 (11%)
Level of Education		
Low n, (%)	35 (44%)	30 (47%)
High n, (%)	44 (56%)	34 (53%)

Table 1b: Characteristics of the EmbryoScope data for the study population, depicted for the first selection (N=79) and after final selection (N=64).

POPULATION	**N=79**	**N=64**
# Oocytes (n)	630	Not applicable
# Embryos (n)	388	Not applicable
Fertilization rate*	62%	Not applicable
Cause subfertility		
Male factor n, (%)	63 (80%)	54 (84%)
Female factor n, (%)	5 (6%)	3 (5%)
Female + Male factor n, (%)	10 (13%)	6 (9%)
Unexplained n, (%)	1 (1%)	1 (2%)
Type of treatment (IVF/ICSI)		
IVF n, (%)	2 (3%)	1 (2%)
ICSI n, (%)	77 (97%)	63 (98%)

* = embryos/oocytes

Table 1c: Table 1b: Characteristics of the SmarterPregnancy data for the study population, depicted for the first selection (N=79) and after final selection (N=64). In the third column, the risk score defined per behavior is displayed.

POPULATION	**N=79**	**N=64**	
Vegetables gr/d			**Risk score**
<150 n, (%)	35 (44%)	28 (44%)	3
150-200 not motivated n, (%)	0 (0%)	0 (0%)	2
150-200 motivated n, (%)	18 (23%)	16 (25%)	1
> 200 (adequate) n, (%)	26 (33%)	20 (31%)	0
Fruits pieces/day			
<1,5 (n, %)	28 (35%)	25 (39%)	3
1,5 - 2 not motivated n, (%)	0 (0%)	0 (0%)	2
1,5 - 2 motivated n, (%)	7 (9%)	6 (9%)	1
> 2 (adequate) n, (%)	44 (56%)	33 (52%)	0
Folic acid			
inadequate n, (%)	1 (1%)	1 (2%)	3
adequate n, (%)	78 (99%)	63 (98%)	0
Smoking			
> 15 n, (%)	0 (0%)	0 (0%)	6
5 tot 15 n, (%)	0 (0%)	0 (0%)	3
1 tot 5 n, (%)	1 (1%)	0 (0%)	1
no smoking n, (%)	78 (99%)	64 (100%)	0
Alcohol			
14+ pw n, (%)	0 (0%)	0 (0%)	3
7 - 14 pw n, (%)	0 (0%)	0 (0%)	2
1 - 7 pw n, (%)	3 (4%)	3 (5%)	1
no drinking n, (%)	76 (96%)	61 (95%)	0
Total			**0 – 18**

3.2 Pre-implantation embryo in relation to nutrition and lifestyle

Table 2 depicts the results of the crude logistic regression model, the results in table 3 are adjusted for the covariate age. The other covariates did not change the OR of the outcome variable KIDScore by > 10% when tested. Table 3 shows a significant negative relation between the NRS and KIDScore (adjusted OR 0,76, CI 0.58 – 0.98, p-value 0,04) and the TRS and the KIDScore (adjusted OR 0.76, CI 0.58 -0.98, p-value 0.04). The relation between vegetables and fruits separate as continue variables and the KIDScore was not significant (vegetables OR 1.01, CI 1.00-1.01, p-value 0.06, fruits OR 1.00, CI 0.99 – 1.01 p-value 0.16). Vegetables as a categorical variable showed a non-significant positive relation with the KIDScore (OR 1.16, CI 0,36 – 3,76, p-value 0.81). Fruits showed a positive relation with the KIDscore (OR 2.76, CI 0.98 – 7.81, p-value 0.06) which was nearly significant.

Table 2: Crude model. Results of logistic regression analysis of the exposure variables NRS, TRS, vegetables and fruits with outcome variable implantation potential categorized as high or low potential, defined by the KIDScore. Results are expressed in an odds ratio (OR), confidence interval (CI) and a p-value.

TABLE 2: CRUDE MODEL	OR	CI	P-VALUE
NRS	0,81	0,63-1,03	0,08
TRS	0,80	0,63-1,03	0,09
Vegetables	1,00	0,99-1,01	0,14
Fruits	1,00	0,99-1,01	0,16
Vegetables inadequate	ref	ref	ref
Vegetables adequate	1	0,32-3,12	1
Fruit inadequate	ref	ref	ref
Fruit adequate	2,88	1,03-8,07	0,04

Table 3: Adjusted model. Results of logistic regression analysis of the exposure variables NRS, TRS, vegetables and fruits with outcome variable implantation potential categorized as high or low potential, defined by the KIDScore. Adjusted for the confounder age. Results are expressed in an odds ratio (OR), confidence interval (CI) and a p-value.

TABLE 3: ADJUSTED MODEL	OR	CI	P-VALUE
NRS	0,76	0,58-0,98	0,04
TRS	0,76	0,58-0,98	0,04
Vegetables	1,01	1,00-1,01	0,06
Fruits	1,00	0,99-1,01	0,16
Vegetables inadequate	ref	ref	ref
Vegetables adequate	1,15	0,35-3,76	0,81
Fruit inadequate	ref	ref	ref
Fruit adequate	2,76	0,98-7,80	0,06

4. Conclusion and discussion

4.1 Conclusion

The research question of this study is: What is the relation between the five SmarterPregnancy characteristics (intake of fruits, intake of vegetables, folic acid supplementation, smoking and use of alcohol) and the implantation potential of a pre-implantation embryo cultured in the EmbryoScope, defined by the KIDscore?

Results show a significant relation between the combined risk scores (NRS and TRS) and the implantation potential of a pre-implantation embryo. The relation found in this study indicates that women with a higher NRS and TRS have a lower chance to develop a high-quality pre-implantation embryo. This association is most likely due to the combination of intake of fruits and vegetables. The other characteristics were insufficient present in this population to be further analyzed.

4.2 Nutrition

Present study's most important findings are that the combined risk scores NRS and TRS are significantly related to the KIDScore. The higher the risk score, indicating unhealthy behavior, the lower the chance of developing an embryo with high implantation potential defined by the KIDScore. This study shows that women undergoing ART treatment have an inadequate intake of fruits (48%) and an inadequate intake of vegetables (69%). These results confirm earlier findings (39-41). The TRS consists of all five SmarterPregnancy characteristics and the NRS consists of three nutritional factors, the intake of fruits, vegetables and folic acid supplementation. The effect of the two combined risk scores marginally differ from each other because three of the five behaviors, smoking, use of alcohol and inadequate folic acid supplementation, were hardly present in the population of this study. Since the established association is almost exclusively found in the nutritional characteristics, in the continuation of this discussion the results of the combined risk scores will be referred to as NRS.

Apparently, intake of fruits and vegetables separately, measured as a continuous variable, are not associated to the implantation potential of the pre-implantation embryo, opposed to fruits and vegetables combined in a risk score. Transforming the variable in a categorical variable by dividing the data in adequate and inadequate intake, the intake of fruits shows an association which is almost significant. For the intake of vegetables, the association is there, although minimal. By analyzing the intake of fruits and vegetables as adequate and inadequate intake, the variables correspond with the nutritional recommendations that may help translating these data into a practical advice. Also, the categorical variables show the effect of adhering to the recommendations on the outcome variable.

Present study's results are in line with the findings of Homan (2007), who observed a relation between nutrition and reproductive health (44). The relation between nutrition and embryonic development is also supported by recent research performed in 2013 by Sinclair (65). To the best of our knowledge, in 2015, Braga was the first to study the effect of nutrition and social behavior on the pre-implantation embryo. She applied traditional morphological assessment of the embryos, with one measurement per day. She found that intake of vegetables and fruits had a significant positive relation with embryo quality. Intake of fruits in particular, was significantly associated to the likelihood of a pre-implantation embryo to form a blastocyst (day 5 embryo) (5).

To study the relation between nutrition and reproductive health, numerous internal and external influences must be taken into account. It is challenging to find research designs in which the many aspects and the development of an infant from the earliest beginning can be captured. Despite these challenges, evidence regarding the relation between nutrition and reproductive health is growing.

Present study is part of that evidence, showing how a combination of nutritional characteristics relates to the implantation potential of a pre-implantation embryo. What this study shows, is that nutrition is significantly associated with the implantation potential of an embryo only three days old. These findings suggest that nutrition in the periconceptional period is associated to embryonic development and thereby possibly to further development of an infant.

4.3 Lifestyle

In the research population of present study, none of the women smoked, only one woman did not use adequate folic acid supplementation and three women used a small amount of alcohol. Recent research by Gormack in New Zealand (2015) and Domar in the USA (2012) on lifestyle choices while undergoing IVF treatment, shows different behavior in their research population (66, 67). Domar designed a prospective study to assess the behavior of women undergoing ART and Gormack studied lifestyle behavior in a cross-sectional study. They both found that indeed some positive changes in behavior took place while undergoing reproductive treatment. However, in both studies during treatment, still half of the women used alcohol. In both studies, no more than 30% of the smokers quit. Both studies conclude that lifestyle advice should be an important part of fertility treatment (66, 67). Why Dutch women undergoing ART in this population have such a healthy lifestyle compared to earlier mentioned studies, remains uncertain. This can be due to good information in the process of treatment in the Erasmus MC, high motivation, due to the fact that this population was highly educated, or maybe some social desirable answers were given.

In this study, only one person did not use adequate amount of folic acid. This is likely for a large part due to the fact that this specific group of women are undergoing ART and are informed about fertility, risks and are motivated to get pregnant. The evidence advocating the use of folic acid in the period of four weeks before until ten weeks of gestation is solid (33, 34). Nevertheless, research by Van der Woude (2012) found that the use of folic acid in the general Dutch population has not increased since 2005. Knowledge about folic acid supplementation in the past few years has actually declined. Van der Woude concluded that 87% of the pregnant women in the study knew about the importance of folic acid use before their pregnancy and only 50% has used folic acid correctly during the entire advised period (68). Research by van der Woude shows that despite all the convincing evidence advocating the use of folic acid, it is not evident that the use of folic acid should be taken for granted. It is without doubt that folic acid supplementation is vital for healthy reproduction, every woman should use folic acid the entire advised period if possible. In practice this means that folic acid should be supplemented when couples are trying to conceive.

The number of participants in the dataset of this study were insufficient or not present at all to assess the association between, smoking and use of alcohol, and the implantation potential of a pre-implantation embryo. In recent literature, relevant findings were done on smoking and the use of alcohol in relation to early embryo development. In 2013, Freour studied the relation between smoking and the early development of embryos using time lapse monitoring. He found that the embryos belonging to women that smoke, developed significant slower and also implantation rates were lower in the group with women that smoked while trying to conceive. This might explain the decline in pregnancy rate in IVF treatment in women that smoke (69). Furthermore, a very recent meta-analysis in 2016 by Joubert et al., found large scale DNA methylations due to maternal smoking. This means that the actual DNA of the newborn has changed due to maternal smoking, presenting possible health risks later in life (70). In 2015, Braga found a negative effect of smoking on the quality of a pre-implantation embryo and a decline in the likelihood that the embryo would develop to blastocyst stage (day-5 embryo) (5). Maternal smoking before and during pregnancy remains an important public health problem that impacts child health in a myriad of ways and has potential lifelong consequences.

Braga also found negative effects for the use of alcohol in the quality of a pre-implantation embryo and for the likelihood of these embryos to develop to blastocyst stage (5). Alcohol has shown to have a direct toxic effect of the developing embryo, the exact mechanisms explaining this effect of alcohol on the embryo are still unknown (71). Altogether, previous reviewed studies indicate that smoking and alcohol use were negatively associated with embryo development.

4.4 Limitations

Because this population is subfertile, it is not possible to generalize the results to the Dutch reproductive population in general. Being subfertile has an effect on people's nutrition and lifestyle. Subfertile women generally have more knowledge about the effects of nutrition and lifestyle on reproductive health. Also, they generally have higher motivation to adopt healthy behavior compared to fertile women, due to the desire of becoming pregnant. This effect is visible in the population of present study. None of the participants smoked, hardly any alcohol is used and all but one supplements folic acid as recommended. In 2017, Van Dijk compared a fertile and an infertile group of couples trying to conceive and studied the chance of conception in relation to nutrition and lifestyle. Data for this research was also collected using the SmarterPregnancy application. The fertile population (N=620) studied in de the research, executed by Van Dijk, showed other numbers for the lifestyle characteristics compared to those of present study. Of the fertile population Van Dijk studied, 11,1% smoked, 32,7% used alcohol and 24,8% didn't use adequate folic acid (43). This means that in the general fertile population, there is a need for information and advice on lifestyle when trying to conceive. Reasons for studying a subfertile population, which is, as discusses different in behavior from the general population, are obvious. It is impossible to do this type of research on reproductive health in the general population. Measuring nutrition and lifestyle around the conception period is hard because the moment of conception is unknown. Capturing images of the embryo inside the uterus is impossible. Findings resulting of research performed on women undergoing fertility treatment can, with precaution, be used to advise fertile couples, surely the principles of reproduction remain the same. The population of present study is different in one other aspect, this study's population has a higher level of education compared to the average level of education in The Netherlands (72). In this study 53% of the participants are highly educated, the Dutch average is 28%.

Another limitation is that 98% of the population underwent ICSI treatment. This is because only the embryos resulting from ICSI treatments were cultured in the EmbryoScope in the Erasmus MC. In ICSI treatments the subfertility is mainly caused by a male factor. In this research, the association between maternal nutrition and lifestyle and the implantation potential of the embryo is studied. However, in this population it may be just as interesting to study the association with paternal nutrition and lifestyle. In 2017, Oostingh published a research about paternal nutrition in relation to semen quality and found an association with healthy diet, especially when semen quality was low. The article abstract concludes with the sentence: "The positive associations between strong adherence to a healthy dietary pattern and semen parameters in men with poor semen quality support the importance of preconceptional tailored nutritional counseling and coaching of couples who are trying to conceive.'' (73). At this moment, the link between semen quality and embryo quality is still unknown.

The data collection on nutrition and lifestyle, derived from the SmarterPregnancy application, is based on self-reporting. This can be a disadvantage because there is no control over the actual data entered. On the other hand, online anonymity is highly valued for it provides a "comfort zone" and encourages honest feedback (74). SmarterPregnancy is an application only available in the Dutch language. For this study, this means that women that did not speak or write Dutch who underwent IVF in the Erasmus MC were not able to participate with SmarterPregnancy.

Furthermore, there is no collection of information on nutrition and lifestyle from the period before the baseline measurements which could be relevant for the study. The data on nutrition collected for this study used former (2006) nutrition guidelines. Also, the analyses on adequate and inadequate intake are according to the former guidelines.
In 2016 new recommendations were formulated (75). Nonetheless this would probably not change the data nor the outcome.
The new recommendation for vegetables is higher than before but since former advice is only met in 31% of the cases, final outcomes would be marginally changed if at all.

Implantation potential may not be the best outcome measure. There is debate about what the best outcome variable to evaluate research on fertility is. Armstrong states that relevant outcomes are livebirth, clinical and ongoing pregnancy and adverse events (29). The problem is that one IVF/ICSI attempt can render several embryos, some of which can be frozen to be used later. This can be several weeks, months or years later. Another reason why outcome variables like live birth ratio are hard to use, is because there are much more other variables that influence the final outcome. Al these other variables will have to be taken into account to deliver reliable results. Due to the complicated and cyclic nature of fertility research, implantation potential was the best feasible outcome measure (29). Another limitation of this research is the relative small population. Furthermore, when randomly selecting one embryo per participant, eight participants were excluded from the total population because none of their oocytes was fertilized, there was no embryo to be selected. Perhaps relevant information for this research got excluded as well, because maybe the reason no oocytes were fertilized, was relevant for this research. The random selection of embryos was necessary to ensure reliability and validity. Subsequently when the KIDscore was assigned to the embryos, seven embryos were excluded that may have added information to this study. Since the KIDscore was the outcome variable, it was essential to assign the KIDScore.

The KIDscore is a result of the most recent developed algorithm, made using the biggest dataset of embryos with known outcome to date. A disadvantage of the KIDScore is that not every embryo with two pronuclei results in a KIDScore. In the original article 95% of embryos with two pronuclei received a KIDScore, in present study this was 87%. It is not clear what the implantation potential of the embryos without the KIDscore is (12).

The risk scores used in this study are carefully thought through and are based on extensive literature studies, assessing the risks involving each behavior. The risk scores have been successfully used in several studies (41, 43) The risk scores enable combining different behaviors in one measure, which enables comparing and analyzing. Nevertheless, the risk scores were not tested or validated before.

Finally, intra and inter observer variability is a known limitation of data collection by observation. In this case, after observing the images provided by the EmbryoScope, the data were annotated in the EmbryoScope database. Variety between observations by different laboratory analysts is possible and can have an effect on the data (53). In this research, the same embryologist checked all the data to minimalize these effects and ensure reliability.

4.5 Strengths

The present study is one of the first to relate nutrition and lifestyle to the implantation potential of a pre-implantation embryo. The first strength of this study is that innovative high quality data, derived from the EmbryoScope was used for this research. The EmbryoScope has only been in use for clinical treatment since 2008 and in the Erasmus since 2012. Time lapse monitoring is a highly promising technique that could change clinical embryology. Although more and bigger RCT's are needed to show effect on clinical pregnancy rates and live births, the data is very valuable for research purposes.

The continuous images of the early development of the embryos provides a treasure of information, that can be used to study embryo development on a level that was impossible before the EmbryoScope was invented.

Secondly, the unique population studied for this research is another strength. Thanks to participants undergoing IVF/ICSI treatment, it is possible to investigate the embryo outside the uterus. The final strength of this study is that the recruits not only participated in the study undergoing fertility treatment, they also cooperated in the SmarterPregnancy study. This made it possible to link the data about the development of the embryos to nutrition and lifestyle behavior of these participants.

4.6 Future research

For future research, more evidence on the relation between nutrition and early embryonic development is needed. Randomized controlled trails are needed to study the effects of the five SmarterPregnancy characteristics, and subsequent suggested measures, have on the development of the pre-implantation embryo. Another aspect that deserves more attention, is the paternal influence. Does intervention on nutrition and lifestyle in men have an effect on embryonic development? And what is the effect if both men and women adjust their nutrition and lifestyle? For this research, the EmbryoScope is an important instrument which will render much more information in the near future.

For future research, it is also evident to study the effects of nutrition and lifestyle related to other outcome measures as suggested in the discussion. What are the clinical implications of an intervention to change couple's nutrition and lifestyle behavior? Will couples in the end hold that baby in their arms? Therefor clinical pregnancy, positive heartbeat and live born babies are desirable. The downside of using these outcomes is that they're much more complicated to measure correctly because of the many other influences that effect these outcomes.

The confirmation that many women do not meet the current recommendations for the intake of fruits and vegetables, even though this research population consist of women with a strong desire to get pregnant, is worrisome. Because of this inadequate intake despite knowledge and efforts to conceive, another aspect of research requires more attention, namely the behavioral aspect. How can important scientific findings about behavior and nutrition, impacting reproductive health, be translated into policies that actually change those detrimental behaviors? In future research on lifestyle, behavior is probably as important as discovering more about the effect of detrimental behaviors. How can behavior like smoking or using alcohol be changed for the benefit of the unborn child and future generations?

Overall, our results show that an increase in the combined risk score NRS decreases the chance of producing an embryo with a high implantation potential defined by the KIDScore. Literature shows similar results. Recent literature also sheds light on the association between embryo development and of the use of alcohol, smoking and inadequate supplementation of folic acid. These behaviors were not analyzed in this population because then were but marginally present. Latest research confirms earlier findings and add evidence to support the advice to refrain from the use of alcohol and cigarettes and take folic acid supplements when contemplating pregnancy.

5. Recommendations

This study shows a relation between nutrition and the implantation potential of a pre-implantation embryo. The relation between lifestyle and the implantation potential could unfortunately not be studied but the conclusions of latest research are evident.

What are clinical implications of these scientific findings? And how can these results eventually be of value for the dietetic practice? This study contributes to insight in early embryonic development. Knowing what is associated with the implantation potential of a pre-implantation embryo helps improving awareness of couples with a desire for a child, and can create more awareness with (reproductive) health professionals. Awareness can help improve conditions to develop embryos with high implantation potential. This research can contribute to the improvement embryonic health of subfertile couples and thereby the success rate of ART can be improved.

In practice this means that couples undergoing fertility treatment should receive comprehensive advice on nutrition and lifestyle. This kind of education is called preconception care (49). Professional advice on nutrition and lifestyle for couples undergoing fertility treatment is explicitly recommended by Braga (2015), Homan (2007), Gormack (2015), Domar (2012), Oostingh (2017) and Van Dijk (2017) (5, 43, 44, 66, 67, 73). Van Dijk (2016) shows that giving advice to couples trying conceive can also be done using a mobile health application. A dietician's contribution is important and self-evident because dieticians are trained to give comprehensive nutritional and lifestyle advice (41).

Eventually knowledge gained by this research can improve awareness and embryonic health of the general population. This research can contribute providing information and educate couples contemplating pregnancy, which are not subfertile. Gynecologists, midwives, general practitioners, dieticians, physiotherapists and psychologists should all contribute in delivering preconception care, in a multidisciplinary way, because many factors are involved in reproductive health. Preconception care should receive more attention and should be made easily available for all couples with a desire to have a child. Hopefully this research can support more health professionals, in particular dieticians, to provide preconception care.

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