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human reproduction

META-ANALYSIS ***Infertility***

Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis

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STUDY QUESTION: Is serum vitamin D associated with live birth rates in women undergoing ART?

SUMMARY ANSWER: Women undergoing ART who are replete in vitamin D have a higher live birth rate than women who are vitamin D deficient or insufficient.

WHAT IS KNOWN ALREADY: Vitamin D deficiency has been associated with an increased risk of abnormal pregnancy implantation as well as obstetric complications such as pre-eclampsia and fetal growth restriction. However, the effect of vitamin D on conception and early pregnancy outcomes in couples undergoing ART is poorly understood.

STUDY DESIGN, SIZE, DURATION: A systematic review and meta-analysis of 11 published cohort studies (including 2700 women) investigating the association between vitamin D and ART outcomes.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: Literature searches were conducted to retrieve studies which reported on the association between vitamin D and ART outcomes. Databases searched included MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and CINAHL. Eleven studies matched the inclusion criteria.

MAIN RESULTS AND THE ROLE OF CHANCE: Live birth was reported in seven of the included studies (including 2026 patients). Live birth was found to be more likely in women replete in vitamin D when compared to women with deficient or insufficient vitamin D status (OR 1.33 [1.08–1.65]). Five studies (including 1700 patients) found that women replete in vitamin D were more likely to achieve a positive pregnancy test than women deficient or insufficient in vitamin D (OR 1.34 ([1.04–1.73]). All 11 of the included studies (including 2700 patients) reported clinical pregnancy as an outcome. Clinical pregnancy was found to be more likely in women replete in vitamin D (OR 1.46 [1.05–2.02]). Six studies (including 1635 patients) reported miscarriage by vitamin D concentrations. There was no association found between miscarriage and vitamin D concentrations (OR 1.12 [0.81–1.54]. The included studies scored well on the Newcastle-Ottawa quality assessment scale.

LIMITATIONS REASONS FOR CAUTION: Although strict inclusion criteria were used in the conduct of the systematic review, the included studies are heterogeneous in population characteristics and fertility treatment protocols.

WIDER IMPLICATIONS OF THE FINDINGS: The findings of this systematic review show that there is an association between vitamin D status and reproductive treatment outcomes achieved in women undergoing ART. Our results show that vitamin D deficiency and insufficiency could be important conditions to treat in women considering ARTs. A randomized controlled trial to investigate the benefits of vitamin D deficiency treatment should be considered to test this hypothesis.

STUDY FUNDING/COMPETING INTERESTS: No external funding was either sought or obtained for this study. The authors have no competing interests to declare.

REGISTRATION NUMBER: N/A.

Key words: Vitamin D / implantation / assisted reproductive treatments / in vitro fertilization / endometrial receptivity

Introduction

Infertility causes great psychological and sometimes physical distress to one in seven couples (National Institute for Health and Care Excellence, 2013). In the UK, in 2014, 52 288 women underwent 67 708 IVF treatment cycles (Human Fertility Embryology Authority, 2016). The overall success rate of these ART was 36.3% (Human Fertility Embryology Authority, 2016). Since the availability of ART treatment has become more widespread, success rates have gradually increased (Grady *et al.*, 2012). This has largely been due to the research conducted in embryology, which has enhanced our abilities to select and transfer the embryo with the highest pregnancy potential. More recently, the rate of improvement in success rates has slowed (Busso *et al.*, 2006). There remains ample room for improvement in fertility treatments to maximize the chances of achieving pregnancy. Much of this lies in improving the likelihood for implantation of the selected embryo that is transferred in to the uterus (Macklon *et al.*, 2002).

There has been recent interest in the role of vitamin D in reproductive physiology as findings have shown that as much as 20–52% of women of reproductive age are deficient in vitamin D (Tangpricha *et al.*, 2002; Gordon *et al.*, 2004; Sullivan *et al.*, 2005). It is postulated that vitamin D is important in the process of pregnancy implantation as vitamin D enzymes and receptors have been found in the endometrium (Lerchbaum & Rabe, 2014). Additionally, vitamin D deficiency has been found to cause decreased fertility capacity, hypogonadism and uterine hypoplasia in animal studies (Halloran & DeLuca, 1980; Kinuta *et al.*, 2000; Yoshizawa *et al.*, 1997; Panda *et al.*, 2001). In humans, the importance of vitamin D in placental function is the most studied aspect of vitamin D in reproduction (Aghajafari *et al.*, 2013). Specifically, vitamin D deficiency has been linked to poor placentation, leading to hypertensive disorders of pregnancy (pre-eclampsia and pregnancy induced hypertension) and fetal growth restriction (Aghajafari *et al.*, 2013). More recently, it has been proposed that vitamin D may be a regulator of initial embryo implantation and that improper implantation, due to vitamin D deficiency, is the cause of poor placentation (Bodnar *et al.*, 2007; Baker *et al.*, 2010; Robinson *et al.*, 2011).

Our main source of vitamin D, a fat-soluble steroid hormone, is from sunlight. Only a small amount is obtained from our diet. The majority of the body's vitamin D is in the form of vitamin D3 (cholecalciferol), which is photo-chemically synthesized in the skin (Holick, 2007).

Vitamin D concentrations are usually measured by assay of serum 25-hydroxy vitamin D_3 status. Experts in nutrition have suggested that people are at risk of the detrimental effects of vitamin D deficiency at serum 25-hydroxy vitamin D_3 concentrations of <50 nmol/l (<20 ng/ml). A concentration of 50–75 nmol/l (21–29 ng/ml) is considered insufficient and greater than 75 nmol/l (greater than 30 ng/ml) is considered vitamin D replete. These vitamin D concentration cut-offs are those adopted by the Endocrine Society (Holick *et al.*, 2011). Differing vitamin D concentration cut-offs have also been proposed by the Institute of Medicine (IOM), who suggest that vitamin D deficiency is when serum 25-hydroxy vitamin D_3 concentrations are <30 nmol/l (<12 ng/ml), vitamin D insufficency is when serum 25-hydroxy vitamin D_3 concentrations are between 30 and 50 nmol/l (between 12 and 20 ng/ml), and that serum 25-hydroxy vitamin D_3 concentrations greater than 50 nmol/l (greater than 20 ng/ml) are considered replete (Ross *et al.*, 2011). There is an agreement that serum concentrations greater than 374 nmol/l (greater than 150 ng/ml) are associated with toxicity and adverse effects (Tangpricha *et al.*, 2002; Heaney, 2008; Stephanou *et al.*, 1994; Daftary & Taylor, 2006).

The biological plausibility that vitamin D plays an important role in implantation has led research groups to investigate the importance of vitamin D in patients undergoing ART. Some studies have found that replete concentrations of vitamin D lead to an increase in clinical pregnancy and live birth rates (Rudick *et al.*, 2012, 2014; Ozkan *et al.*, 2010; Garbedian *et al.*, 2013; Paffoni *et al.*, 2014). However, others have found conflicting evidence suggesting that vitamin D has no effect on the outcome of ART(Anifandis *et al.*, 2010; Aleyasin *et al.*, 2011; Firouzabadi *et al.*, 2014; Fabris *et al.*, 2014; Franasiak *et al.*, 2015). The aim of our review was to investigate the association between vitamin D status and reproductive outcomes by meta-analysis of the ART outcomes of published cohort studies to summarize the available evidence.

Materials and Methods

Inclusion criteria

The study was designed *a priori* with inclusion of primary articles that studied women undergoing any form of ART (IVF, ICSI and frozen embryo transfer [FET]) who had their vitamin D status checked. This could either be through blood serum or follicular fluid assay. The primary outcome was live birth rates according to vitamin D status. Secondary outcomes included biochemical pregnancy rates, and clinical pregnancy rates.

Literature search

MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and CINAHL (from inception to April 2017) were searched. The search strategy used the following key words and/or medical subject heading (MeSH) terms: pregnancy, IVF, intracytoplasmic sperm injection, ART and vitamin D. The full electronic search strategy is provided in Supplementary Table SI. References of all included primary and review articles were examined to identify relevant articles not captured by the electronic searches. No language restrictions were applied in any of the searches or study selection.

Study selection

Criteria for inclusion in the study were established prior to the literature search. Two independent reviewers (J.C. and B.T.) carried out study selection. First, the independent reviewers scrutinized the titles and abstracts of the electronic searches. Each title and abstract were included or excluded independently according to the predefined inclusion criteria; any disagreements regarding inclusion were resolved by a further reviewer (I.D.G). The full manuscripts of the titles and abstracts considered to be relevant

for inclusion were obtained. When there was a duplicate publication, the most recent and complete version was selected and included. Studies that did not explicitly report results from ARTs according to vitamin D groups (deficient, insufficient and replete) according to Endocrine Society guidelines were excluded.

The same two independent reviewers (J.C. and B.T.) extracted the outcome data from the included studies.

Study quality assessment

Two reviewers (J.C and B.T.) used the Newcastle-Ottawa Quality Assessment Scales for observational studies to complete a quality assessment of the included manuscripts (Wells *et al.*, 2011). The Newcastle-Ottawa scale ranges from zero to nine, awarding one star for all categories (case-cohort representative, ascertainment of exposure, outcome negative at commencement of study, outcome assessment, duration of follow-up and adequacy of follow-up) except comparability by design or analysis where two stars can be awarded. An arbitrary score was allocated assuming that all items have equal weighting. This was used to give a quantitative appraisal of overall quality of the individual studies. Each study received a score from each of the reviewers.

Publication bias

Assessment for publication bias in the included studies for the outcome of clinical pregnancy was performed using Harbord's modified test for small study effects to assess for funnel plot asymmetry (Harbord *et al.*, 2006).

Statistical analysis

Live birth, biochemical pregnancy, clinical pregnancy and miscarriage rates were extracted from each of the included studies according to vitamin D strata. The log of the ratio and its corresponding standard error for each study was computed. Meta-analysis using inverse-variance weighting was performed to calculate the random-effects summary estimates. The square root of this number is the estimated standard deviation of the underlying effects across studies. Because, we had relative measures of effect, the CIs were centered on the natural logarithm of the pooled estimate and the limits exponentiated to obtain an interval on the ratio scale. Forest plots were created for each outcome, showing individual study proportions with CIs and the overall DerSimmonian-Laird pooled estimate according to vitamin D status. Heterogeneity of the treatment effects was assessed graphically with forest plots and statistically analyzed using the χ^2 test. Statistical analyses were performed using Stata 12.1 (StataCorp, College Station, TX, USA).

Results

The PRISMA flow diagram (Liberati *et al.*, 2009; Moher *et al.*, 2009) of the review process is presented in Fig. 1. The search strategy yielded 4615 citations, of which 4505 citations were excluded as it was clear from scrutinizing the title and abstract that they did not fulfill the selection criteria. Full manuscripts of 110 articles were obtained. A total of 99 of these publications were excluded because 35 were reviews, 24 articles did not specify outcomes from ART, 17 articles did not specify investigating vitamin D, seven articles were conference abstracts or studies where there was no extractable data (Farzadi *et al.*, 2015; Neville *et al.*, 2016) (as they provided mean vitamin D concentrations of groups of women achieving clinical pregnancy and those that did not), five articles reported male infertility, four articles were animal studies, three were letters, two were duplicates, and one was a study protocol. Therefore, the total number of observational studies included in the review was 11.

Study characteristics

Study characteristics of the 11 included studies are presented in Table I. None of the included studies declared any conflicts of interest. The included studies varied in publication date between 2010 and 2015. All 11 included studies were cohort studies; 6 were retrospective and 5 were prospective in design. Sample sizes varied between 84 women to 517 women. Nine of the 11 included studies reported the ages of their study population. Seven studies had a mean age of below 37 years and two had a higher mean age of 40.5 and 40.9 years. Eight included studies used serum measurement of vitamin D, two used both follicular fluid and serum vitamin D (finding that there was high correlation between the follicular fluid vitamin D and serum vitamin D in their participants), and one study used follicular fluid alone. Of the 11 included studies, nine studies reported ART where women had used autologous oocytes. Two reported results from women who were donor egg recipients. One study used pre-implantation genetic screening to ensure that patients had karyotypically normal embryos transferred. One study chose to only study women that underwent a single blastocyst transfer. All of the 11 included studies assayed 25-hydroxy-vitamin D. Four of the included studies assessed vitamin D before the commencement of the treatment cycle, three assessed vitamin D at the time of ovulation trigger, three assessed vitamin D at the time of oocyte retrieval, and one study assessed vitamin D just before oocyte retrieval. All of the 11 included studies used the Endocrine Society classification of vitamin D status (<50 nmol/l deficient, 50–75 nmol/l insufficient and greater than 75nmol/l replete). Six of the included studies provided adjusted odds ratios, adjusting for potential confounding factors. Of these six studies, only four provided adequate detail for potential meta-analysis of adjusted odds ratios. However, two of these studies had adjusted for vitamin D concentration and another two studies had used differing referent groups to obtain adjusted odds ratios.

A funnel plot to test for asymmetry did not find substantial evidence of publication bias ($P = 0.933$) (Supplementary Fig. S1).

All studies scored well using the Newcastle-Ottawa Quality Assessment achieving a score between 7 and 9 (Table II).

Vitamin D deficiency prevalence

Our review found a high prevalence of vitamin D deficiency. The meta-analyzed prevalence for vitamin D deficiency, insufficiency and replete were 34.6% (95% CI 32.0–37.4), 45.3% (95% CI 42.4–48.5) and 25.7% (95% CI 23.4–28.2%), respectively.

Live birth

Seven studies (2026 participants) reported the live births achieved by women when categorized by vitamin D (Fig. 2). Meta-analysis of the data from these studies showed that women who are vitamin D replete have a higher chance of achieving a live birth from ART when compared with women with vitamin D deficiency or insufficiency. The odds ratio was 1.33 (1.08–1.65). The meta-analysis had low statistical heterogeneity with an I^2 value of 5.0% ($P = 0.39$).

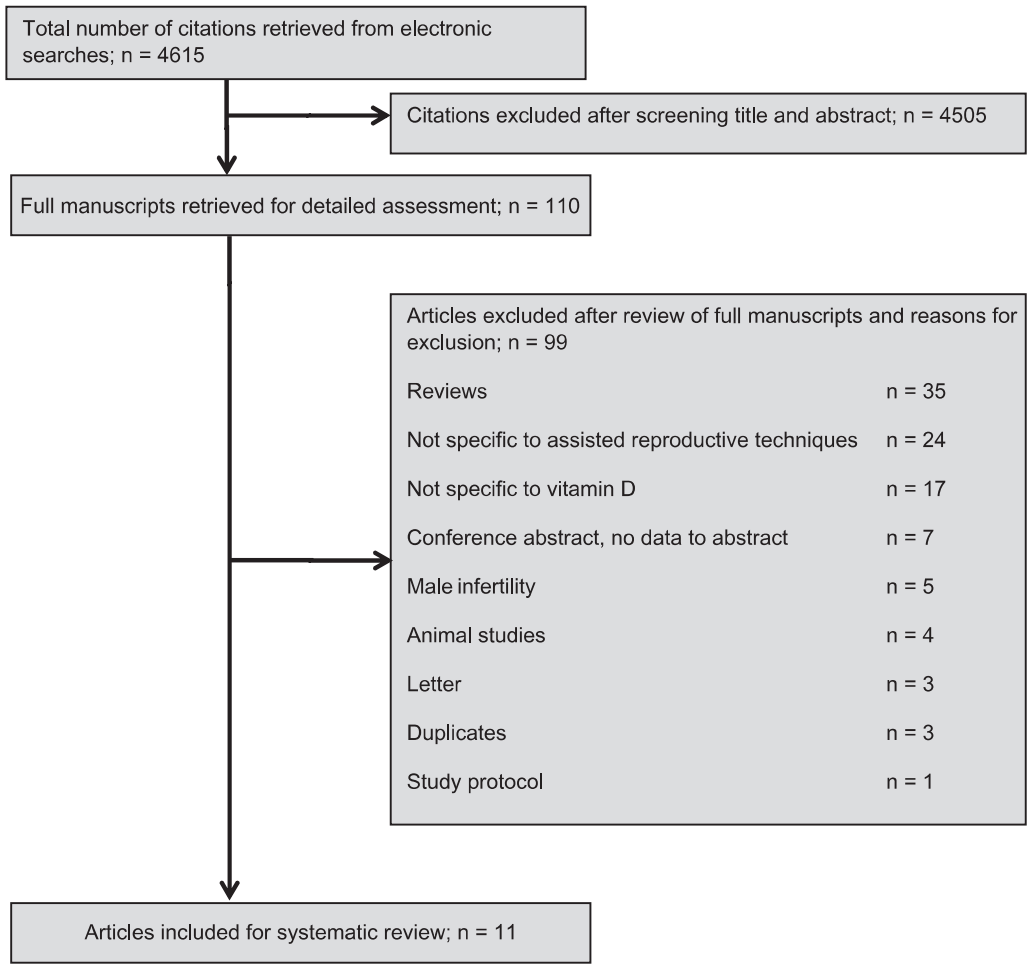

Figure 1 PRISMA flow diagram for study selection.

Biochemical pregnancy

Five studies (1700 participants) reported the number of women that achieved a positive pregnancy test approximately two weeks after embryo transfer for the three vitamin D categories. The odds of biochemical pregnancy in the vitamin D deficient and insufficient population versus the vitamin D replete population are presented in Fig. 3. Meta-analysis of these five cohort studies showed a greater chance of pregnancy in the vitamin D replete group when compared with the vitamin D deficient and insufficient groups with an odds ratio of 1.34 (1.04–1.73). There was a low level of statistical heterogeneity with an I^2 value of 21.0% ($P = 0.28$).

Clinical pregnancy

All 11 studies (2700 participants) reported on clinical pregnancy rate (the presence of fetal heart approximately five weeks after embryo transfer) as an outcome (Fig. 4). Pooling of the clinical pregnancy outcomes from the 11 studies showed an improved chance of clinical pregnancy in the vitamin D replete population when compared with the vitamin D deficient and insufficient population. The vitamin D replete group was more likely to achieve clinical pregnancy when compared with the vitamin D deficient and insufficient groups with an odds ratio of 1.46 (1.05–2.02). The I^2 value for this meta-analysis was 61.0% suggesting a moderate level of statistical heterogeneity ($P = 0.02$).

Data could be extracted from nine of the included studies (2082 patients) to compare the chances of clinical pregnancy by using the IOM definitions of vitamin D status (vitamin D concentrations of <50 nmol/l considered as deficient or insufficient and vitamin D concentrations of more than 50 nmol/l considered replete). Pooling of the clinical pregnancy rates from these nine studies also showed that women with a vitamin D concentration of greater than 50 nmol/l were more likely to achieve a clinical pregnancy when compared to women with a vitamin D concentration of below 50 nmol/l with an odds ratio of 1.38 (1.04–1.83) (Supplementary Fig. S2).

Clinical pregnancy according to source of oocyte used

The 11 included studies were divided into two groups according to the source of the oocyte (autologous or donor) used to form the embryo

Table I Characteristics of included studies

Author (year)	Study design	Study population	Age of study population	Bio-fluid used for vitamin D assessment	Timing of vitamin D assessment	Method of vitamin D assessment	Vitamin D cut-offs utilized	Autologous or donated oocyte	Summary of results		Confounders adjustment	Conclusions
Anifandis *et al.* (2010)	Prospective Cohort	101 women undergoing IVF in Greece	Not reported	Vitamin D in follicular fluid	At oocyte retrieval	25-OH vitamin D by electrochemiluminescence immunoassay (ECLIA)	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen 3-4 weeks on ultrasound scan post-HCG) 10/31 deficient group 16/49 insufficient group 3/21 replete group Pregnancy test positive Data not provided	Miscarriage Data not provided Live birth Data not provided	Nil	Follicular fluid vitamin D concentrations significantly correlated to the quality of the embryos. Data suggested that high concentrations of vitamin D led to a decreased chance of clinical pregnancy
Fabris *et al.* (2014)	Retrospective Cohort	267 women undergoing donor oocyte IVF in Spain	Mean age 40.5 years	Vitamin D in serum	At oocyte retrieval	25-OH vitamin D by ELISA	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/L	Donated	Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer) 68/92 deficient group 94/134 insufficient group 29/41 replete group Pregnancy test positive (pregnancy test positive 2 weeks after embryo transfer) 60/92 deficient group 85/134 insufficient group 25/41 replete group	Miscarriage (pregnancy loss after clinical pregnancy achieved) 8/92 deficient group 9/134 insufficient group 4/41 replete group Live birth 56/92 deficient group 71/134 insufficient group 23/41 replete group	Nil	No significant difference in implantation or clinical pregnancy rates between deficient, insufficient and replete vitamin D groups

Continued

Table I *Continued*

Author (year)	Study design	Study population	Age of study population	Bio-fluid used for vitamin D assessment	Timing of vitamin D assessment	Method of vitamin D assessment	Vitamin D cut-offs utilized	Autologous or donated oocyte	Summary of results		Confounders adjustment	Conclusions
Firouzabadi *et al.* (2014)	Prospective Cohort	221 women undergoing IVF in Iran	Mean age 29.2 years	Vitamin D in follicular fluid and serum	At oocyte retrieval	25-OH vitamin D by ELISA	Deficiency <25 nmol/l Insufficiency 25–75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined]) 23/50 deficient group 47/155 insufficient group 4/16 replete group Pregnancy test positive Data not provided	Miscarriage Data not provided Live birth Data not provided	Nil	No significant correlation between follicular fluid or serum vitamin D and clinical pregnancy rate. Significant correlation between follicular fluid vitamin D concentrations and serum vitamin D concentrations
Franasiak *et al.* (2015)	Retrospective cohort	517 women undergoing IVF with euploid blastocyst transfer in USA	Mean age 35.0 years	Vitamin D in serum	At ovulation trigger injection	25-OH vitamin D by ELISA	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined]) 144/206 deficient group 151/215 insufficient group 64/96 replete group Pregnancy test positive (pregnancy test positive 2 weeks after embryo transfer)163/206 deficient group 162/215 insufficient group 74/96 replete group	Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen or pregnancy loss after gestational sac seen) 32/206 deficient group 29/215 insufficient group 14/96 replete group Live birth 131/206 deficient group 133/215 insufficient group 60/96 replete group	Adjustment for age, BMI, ethnicity, season, number of previous treatment cycles, number of embryos transferred	Vitamin D status unrelated to pregnancy rates in women undergoing euploid blastocyst transfers

Fru *et al.* (2014)	Retrospective Cohort	102 women undergoing IVF in USA	Not reported	Vitamin D in serum	Pre-cycle but not defined	25-OH vitamin D Method not defined	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (not defined) 6/18 deficient group 24/47 insufficient group 37/58 replete group Pregnancy test positive Data not provided	Miscarriage (not defined) 1/6 deficient group 9/24 insufficient group 12/37 replete group Live birth 5/18 deficient group 15/47 insufficient group 25/58 replete group	Nil	Higher vitamin D concentrations correlated with increased likelihood of positive pregnancy test. Overall live birth rates highest in vitamin D replete group
Garbedian *et al.* (2013)	Prospective Cohort	173 women undergoing IVF in Canada	Mean age 34.5 years	Vitamin D in serum	Before oocyte retrieval	25-OH vitamin D Method not defined	Deficiency and insufficiency <75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined]) 33/95 deficient and insufficient groups combined 41/78 replete group Pregnancy test positive Data not provided	Miscarriage Data not provided Live birth Data not provided	Adjustment for age, BMI, number of embryos transferred and vitamin D concentration	Implantation and clinical pregnancy rates are higher in the vitamin D sufficient group (>75 nmol/l). Statistical significant difference in clinical pregnancy rate, no statistical difference in pregnancy positive rate
Ozkan *et al.* (2010)	Prospective Cohort	84 women undergoing IVF in Turkey	Mean age 34.4 years	Vitamin D in follicular fluid and serum	At ovulation trigger injection	25-OH vitamin D Method not defined	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined]) 5/23 deficient	Miscarriage Data not provided Live birth Data not provided	Adjustment for age, BMI, ethnicity, number of embryos transferred and vitamin D concentration	Serum and follicular fluid strong correlated. Higher implantation and clinical pregnancy rates in insufficient (20–

Continued

Table I *Continued*

Author (year)	Study design	Study population	Age of study population	Bio-fluid used for vitamin D assessment	Timing of vitamin D assessment	Method of vitamin D assessment	Vitamin D cut-offs utilized	Autologous or donated oocyte	Summary of results		Confounders adjustment	Conclusions
									group 6/30 insufficient group 15/31 replete group Pregnancy test positive (pregnancy test positive 2 weeks after embryo transfer) 3/23 deficient group 4/30 insufficient group 8/31 replete group			30ng/ml) and replete concentrations (>30ng/ml) when compared to deficient group-highest in replete group
Paffoni *et al.* (2014)	Prospective cohort	335 women undergoing IVF in Italy	Mean age 36.9 years	Vitamin D in serum	Pre-cycle but not defined	25-OH vitamin D by electrochemiluminescence immunoassay (ECLIA)	Deficiency <50 nmol/l Insufficiency50–75 nmol/l Replete>75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined]) 30/154 deficient group 33/117 insufficient group 23/64 replete group Pregnancy test positive (pregnancy test positive 2 weeks after embryo transfer) 34/154 deficient group 36/117 insufficient group 25/64 replete group	Miscarriage Data not provided Live birth 29/154 deficient group 28/117 insufficient group 19/64 replete group	Nil	Analysis suggested those with a vitamin D >75nmol/l had the highest chance of clinical pregnancy when compared with those with vitamin D deficiency or insufficiency

Polyzos *et al.* (2014)	Retrospective cohort	368 women undergoing IVF resulting in single blastocyst embryo transfer in Belgium	Mean age 30.6 years	Vitamin D in serum	At ovulation trigger injection	25-OH vitamin D by ELISA	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer) 98/239 deficient group 70/129 insufficient and replete group combined Pregnancy test positive (pregnancy test positive 2 weeks after embryo transfer) 124/239 deficient group 86/129 insufficient and replete groups combined	Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen or pregnancy loss after gestational sac seen) 44/239 deficient group 25/129 insufficient and replete groups combined Live birth 78/239 deficient group 61/129 insufficient and replete groups combined	Adjustment for age, number of previous treatment cycles, type of treatment protocol. Type of gonadotrophin used, starting dose of gonadotrophin, E2 levels on day of HCG, number of oocytes collected, type of treatment, day 5 embryo transfer, top quality embryo transfer, endometrial thickness, serum progesterone at trigger injection, season and vitamin D concentration	Clinical pregnancy rate significantly lower in vitamin D deficient group $P = 0.015$. Controlled for 16 confounding factors
Rudick *et al.* (2012)	Retrospective cohort	188 women undergoing IVF in USA	Mean age 36.0 years	Vitamin D in serum	Pre-cycle but not defined	25-OH vitamin D by ELISA	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/l	Autologous	Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer) 14/39 deficient	Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen or pregnancy loss after gestational sac	Adjustment for age, number of embryos transferred, embryo quality and diagnosis of diminished ovarian reserve	Vitamin D deficiency associated with lower CPR in non-hispanic whites but not in Asians

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Table I *Continued*

Author (year)	Study design	Study population	Age of study population	Bio-fluid used for vitamin D assessment	Timing of vitamin D assessment	Method of vitamin D assessment	Vitamin D cut-offs utilized	Autologous or donated oocyte	Summary of results		Confounders adjustment	Conclusions
									group 29/70 insufficient group 34/79 replete group Pregnancy test positive Data not provided	seen) 3/39 deficient group 7/70 insufficient group 8/79 replete group Live birth 11/39 deficient group 22/70 insufficient group 26/79 replete group		
Rudick *et al.* (2014)	Retrospective cohort	99 women undergoing donor oocyte IVF in USA	Mean age 40.9 years Range 21–39	Vitamin D in serum	Pre-cycle but not defined	25-OH vitamin D by ELISA	Deficiency <50 nmol/l Insufficiency 50–75 nmol/l Replete >75 nmol/l	Donated	Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer) 9/26 deficient group 16/38 insufficient group 26/35 replete group Pregnancy test positive Data not provided	Miscarriage (pregnancy loss after positive pregnancy test but before intrauterine gestational sac seen or pregnancy loss after gestational sac seen) 1/26 deficient group 3/38 insufficient group 6/35 replete group Live birth 8/26 deficient group 13/38 insufficient group 20/35 replete group	Adjustment for embryo quality, BMI and ethnicity	Lower CPRs in those with vitamin D deficiency suggesting that the effects are localized within the endometrium

Table II Newcastle-Ottawa Scale appraisal of included studies

Study	Case representative	Control representative	Ascertainment of exposure	Outcome negative at start	Comparability by design or analysis	Outcome assessment	Duration of follow-up	Adequacy of follow-up	Score
Anifandis *et al.* (2010)	*	*	*	*	**	*	*	x	8
Fabris *et al.* (2014)	*	*	*	*	*	*	*	*	8
Firouzabadi *et al.* (2014)	*	*	*	*	x	*	*	*	7
Franasiak *et al.* (2015)	*	*	*	*	*	*	*	*	8
Fru *et al.* (2014)	*	*	*	*	x	*	*	*	7
Garbedian *et al.* (2013)	*	*	*	*	*	*	*	*	8
Ozkan *et al.* (2010)	*	*	*	*	**	*	*	*	9
Paffoni *et al.* (2014)	*	*	*	*	*	*	*	*	8
Polyzos *et al.* (2014)	*	*	*	*	**	*	*	*	9
Rudick *et al.* (2012)	*	*	*	*	x	*	*	*	7
Rudick *et al.* (2014)	*	*	*	*	**	*	*	*	9

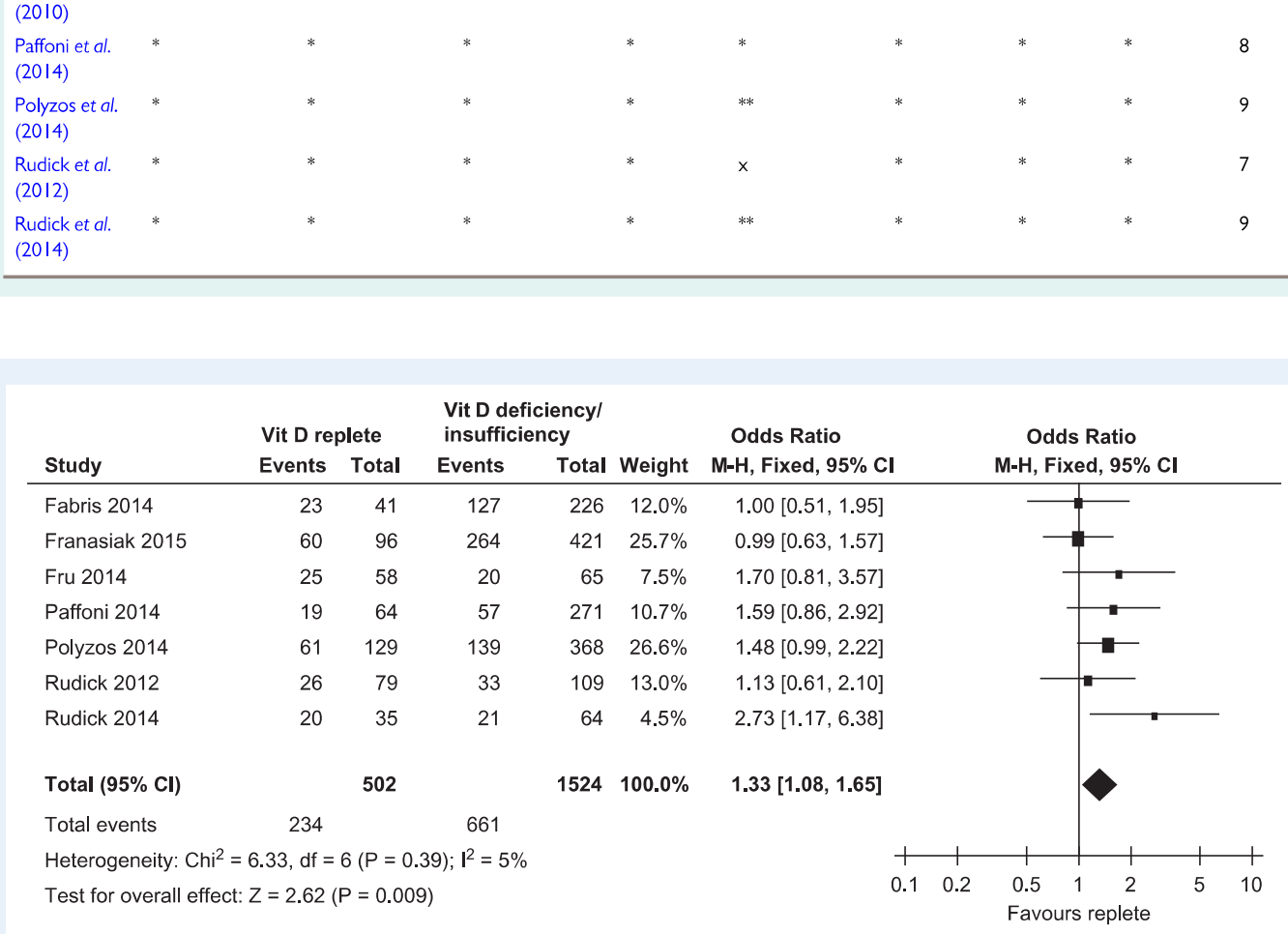

Figure 2 Meta-analysis of studies reporting live birth by vitamin D concentrations. Meta-analysis of the data from seven included studies that reported live birth as an outcome showed that women who are vitamin D replete have a higher chance of achieving a live birth from ART when compared with women with vitamin D deficiency or insufficiency. F-H, Fixed; Fixed effects (Mantel-Haenszel).

for transfer (Fig. 5). Nine studies (including 2334 patients) reported fertility outcomes in infertile women receiving an autologous oocyte embryo. Clinical pregnancy was found to be more likely in women who were vitamin D replete who received an autologous oocyte embryo (OR 1.39 [1.00–1.93]). The I^2 value for this meta-analysis was 56.0% suggesting a moderate level of statistical heterogeneity ($P = 0.02$).

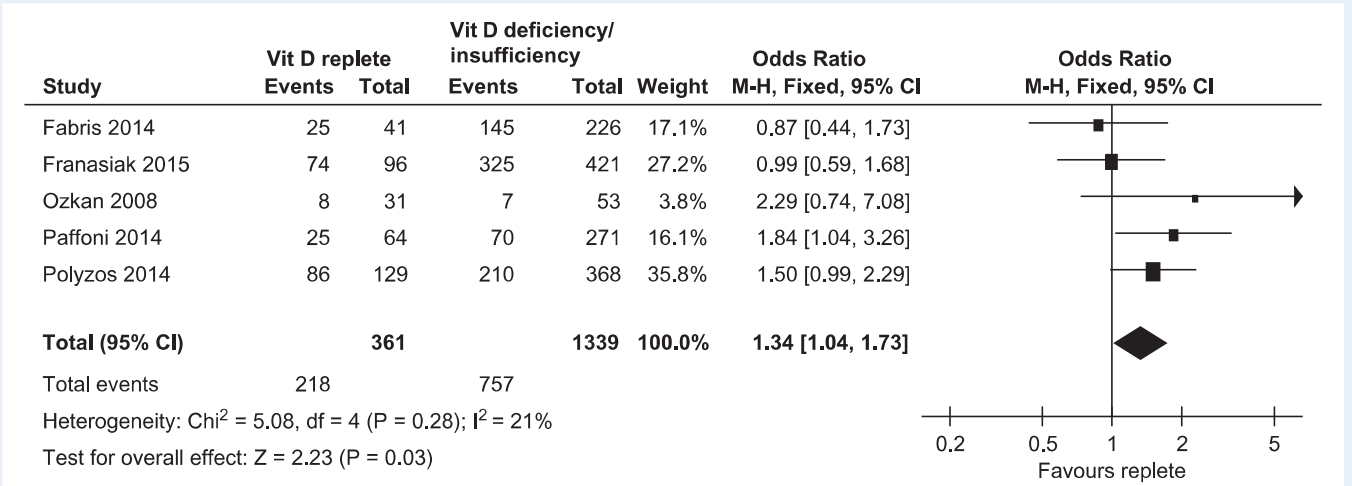

Figure 3 Meta-analysis of studies reporting biochemical pregnancy by vitamin D concentrations. Meta-analysis of the data from five included studies that reported biochemical pregnancy as an outcome showed that women who are vitamin D replete have a higher chance of achieving a positive pregnancy test from ART when compared with women with vitamin D deficiency or insufficiency.

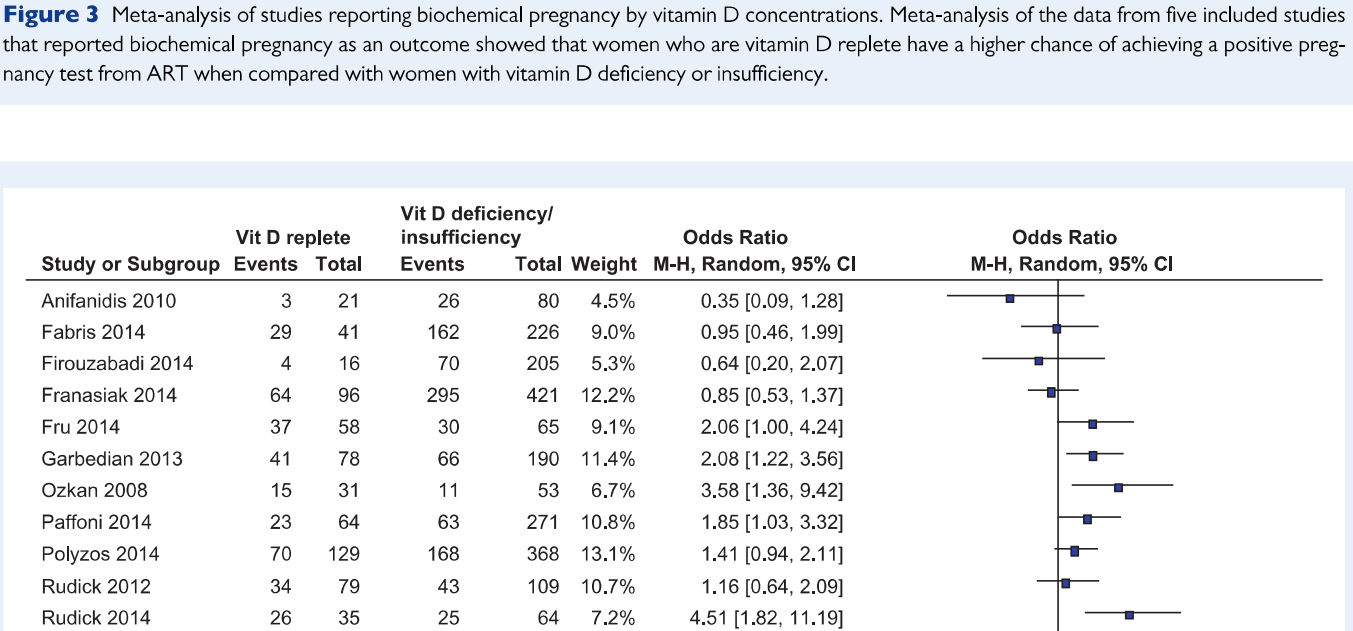

Figure 4 Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations. Meta-analysis of the data from all 11 of the included studies that reported clinical pregnancy as an outcome showed that women who are vitamin D replete have a higher chance of achieving clinical pregnancy from ART when compared with women with vitamin D deficiency or insufficiency.

In the two studies (including 366 patients) where women received a donor oocyte embryo, no significant difference was found when comparing the clinical pregnancy in women receiving a donor oocyte embryo who were vitamin D replete when compared to women who were vitamin D deficient or insufficient (OR 2.02 [0.44–9.26]). The I^2 value for this meta-analysis was 85.0% suggesting a considerable level of statistical heterogeneity ($P = 0.009$).

Miscarriage

Six studies (1635 participants) reported on the outcome of miscarriage (Fig. 6). When the data from these six studies are pooled, the chance of miscarriage in the vitamin D replete women is similar to that of vitamin D deficient and insufficient women with an odds ratio of 1.12 (0.81–1.54). There was a low level of statistical heterogeneity denoted by an I^2 value of 0.0% ($P = 0.76$).

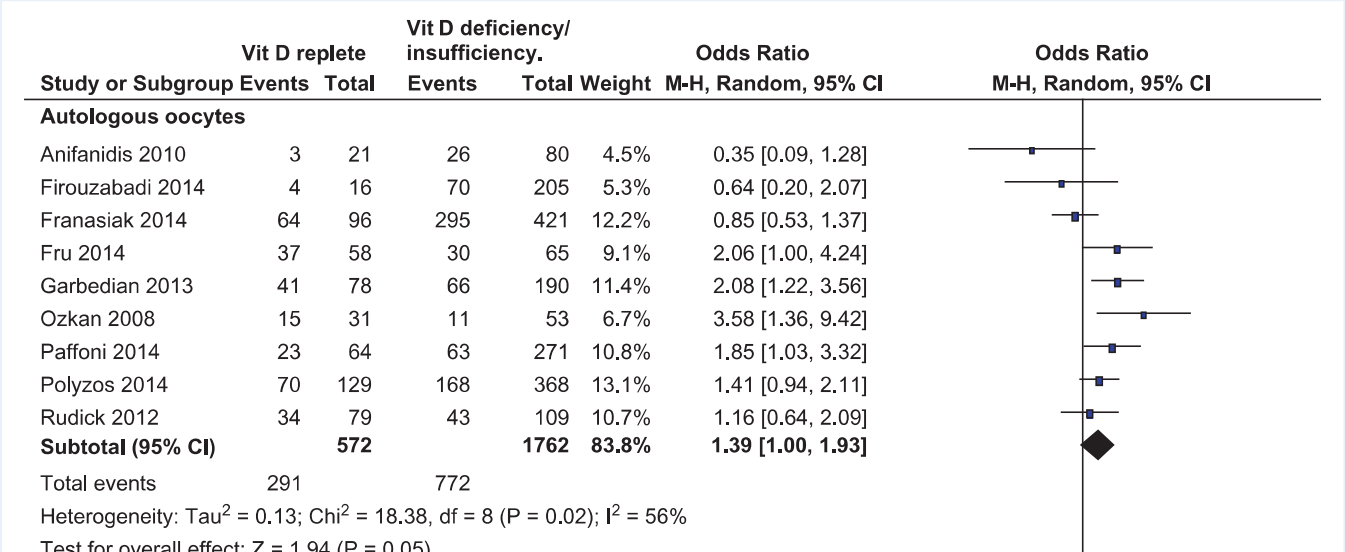

Figure 5 Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations according to source of oocyte. Meta-analysis of the data from nine included studies showed that women who are vitamin D replete have a higher chance of achieving a clinical pregnancy from ART using autologous oocytes when compared with women with vitamin D deficiency or insufficiency. Meta-analysis of the data from two included studies showed no difference in the chance of clinical pregnancy in women replete, insufficient or deficient in vitamin D undergoing ART using donor oocytes.

Discussion

This systematic review including 11 studies suggests that the chances of achieving a live birth, a positive pregnancy test and clinical pregnancy after ART are higher in women who are vitamin D replete when compared to those who are vitamin D deficient or insufficient. Miscarriage does not appear to be associated with vitamin D status.

Our analysis was strengthened by a number of factors. A comprehensive search strategy was used, employing relevant research databases. Additionally, a valid data synthesis method was implemented and no language restrictions were applied. The Newcastle-Ottawa Quality Assessment Scale was used to assess the quality of the included studies. The assessment of all studies scored well on this scale, suggesting low risk of bias.

There are also weaknesses in our analysis, which mainly stem from the clinical heterogeneity of the publications that were included. Some degree of heterogeneity is to be expected due to the different geographical locations that the individual cohort studies have been conducted, leading to differing population characteristics and ART protocols used. However, this is not necessarily a disadvantage as some degree of clinical heterogeneity can increase the generalizability of the findings to wider infertility populations.

Ideally, when meta-analyzing cohort studies, the adjusted odds ratios (where provided) should be meta-analyzed. However, in our included studies it was infrequent for the included primary studies to have provided sufficient detail of their adjusted analysis for known confounding factors such as age and BMI. Therefore, we were unable to perform a meta-analysis of adjusted odds ratios.

One source of clinical heterogeneity between the included studies is in the timing of vitamin D assessment. Some of the studies measured their participants' vitamin D status before the start of ART, whereas others measured vitamin D at the time of oocyte retrieval. Vitamin D status is known to not fluctuate over time unless vitamin D deficiency or insufficiency is actually treated (Anagnostis *et al.*, 2013). Therefore, the importance of the difference in timing of the vitamin D assessment reduces.

There were also differences in the bio-fluid used to assess vitamin D status amongst the included studies. Three of the included studies measured vitamin D in the follicular fluid aspirated at the time of oocyte retrieval. The remaining studies used blood serum for vitamin

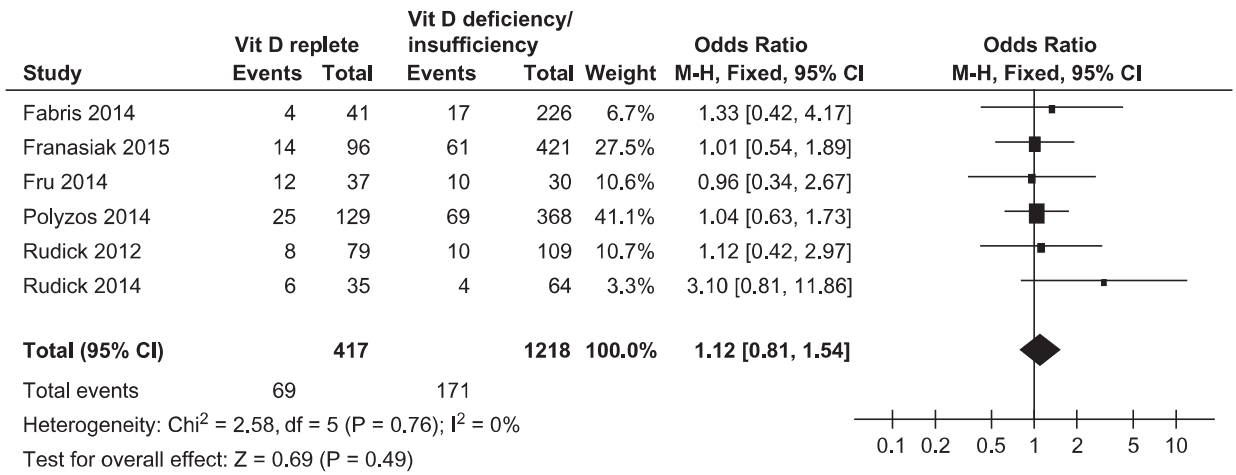

Figure 6 Meta-analysis of studies reporting miscarriage by vitamin D concentrations. Meta-analysis of the data from six included studies that reported miscarriage as an outcome showed no difference in the chance of miscarriage in women replete, insufficient or deficient in vitamin D undergoing ART.

D measurement. Reassuringly, a number of previously published studies have found that assays of vitamin D in follicular fluid or blood serum produce results that are highly correlative (Aleyasin *et al.*, 2011; Anifandis *et al.*, 2010; Firouzabadi *et al.*, 2014; Ozkan *et al.*, 2010). Serum vitamin D would be measured more conveniently in women undergoing ART and could be tested before the start of treatment to allow time for correction of deficiency.

We found that the likelihood of achieving a positive pregnancy test after embryo transfer was higher in women who were replete in vitamin D. This would support the hypothesis that vitamin D affects embryo implantation. Two of the included studies have tried to investigate the effect of vitamin D on implantation further by only including women undergoing oocyte recipient treatment cycles (Fabris *et al.*, 2014; Rudick *et al.*, 2014). Isolating recipients of donor oocyte embryos aims to reduce the impact of oocyte quality on reproductive outcomes. Donated oocytes would be sourced from younger women with higher quality oocytes and therefore implantation can be investigated more accurately. Meta-analysis of the clinical pregnancy data from these two studies (including 366 patients) did not show a statistically significant difference in chance of clinical pregnancy between the vitamin D replete and vitamin D deficient or insufficient populations. However, the data may suggest a higher chance of clinical pregnancy in the vitamin D replete group. It is likely that the failure to reach statistical significance is due to the low number of participants in view of the wide CIs (Cochrane Collaboration, 2011). Removal of these two studies from the overall analysis did not alter the overall association between vitamin D concentration and clinical pregnancy.

Seasonal variations in conception rates have been established (Rojansky *et al.*, 1992) with higher conception rates found in the Summer and Autumn. Although many hypotheses have been postulated to explain this phenomenon (e.g. reduced ovulation rates and poorer sperm quality in darker months) the exact mechanism behind this has not been explained. It is possible that an increase in sun exposure and greater sunlight luminosity increases the body's store of vitamin D, thereby yielding higher conception rates in Summer and Autumn.

Although, the debate regarding the importance of vitamin D and seasonal variation in reproductive health continues, its impact on immunomodulation within the endometrium with a resultant reduction in active inflammatory cytokines is now well understood (Holick, 2007). The expression of vitamin D receptors at the level of the endometrium and the role of vitamin D in the transcription of HOX10A gene (found to be of key importance in implantation) suggest that the immunomodulatory effects of vitamin D may have a direct impact on implantation and therefore the likelihood of reproductive treatment success (Evans *et al.*, 2004).

Ethnicity has also been found to be a prognostic marker for IVF treatment success, with women of Asian and Black ethnic origins having worse reproductive outcomes (Dhillon *et al.*, 2016). One possible explanation for this finding could be lower serum vitamin D concentrations in these ethnic groups or differences in the vitamin D receptor gene polymorphisms (Ingles, 2007; John *et al.*, 2007).

Our review demonstrates that replete vitamin D status is associated with greater chances of ART success. This could be via the actions of vitamin D on the endometrium promoting embryo implantation or as a surrogate marker for general well-being (Lerchbaum & Rabe, 2014). Vitamin D serum testing is relatively cheap and widely available and its treatment is not costly. Therefore, it may be beneficial to diagnose and treat vitamin D deficiency in women planning ART to optimize their pregnancy outcomes. Correction of vitamin D deficiency in these patients would also be of benefit during pregnancy, as replete vitamin D concentrations have been found to reduce the risk of obstetric complications such as gestational diabetes (Wang *et al.*, 2012; Zhang *et al.*, 2015), pre-eclampsia (Moon *et al.*, 2015; De-Regil *et al.*, 2012; Wei, 2014) and fetal growth restriction (Conde-Agudelo *et al.*, 2013; Khalessi *et al.*, 2015). To further investigate the value of treatment of vitamin D deficiency in the infertile population an interventional trial would be necessary.

Supplementary data

Supplementary data are available at *Human Reproduction online*.

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Authors' roles

J.C. and A.C. were responsible for defining the research question. J.C. designed the strategy for literature search. J.C. and B.T. assessed eligibility of studies for inclusion to the systematic review. Statistical analyses were performed by A.T. and IDG. A.E. assisted in the design of the systematic review search strategy and in manuscript preparation. J.C. wrote the first draft of the manuscript and is its guarantor. All authors revised it critically for important intellectual content and gave final approval of the version to be published.

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Conflict of interest

None declared.

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