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

Gallos, Ioannis; Chu, Justin; Tobias, Aurelio; Tan, Bee; Eapen, Abey; Coomarasamy, Aravinthan

DOI:
10.1093/humrep/dex326

Document Version
Peer reviewed version

Citation for published version (Harvard):
Gallos, I, Chu, J, Tobias, A, Tan, B, Eapen, A & Coomarasamy, A 2017, 'Vitamin D and assisted reproductive treatment outcome: A systematic review and meta-analysis', *Human Reproduction*, vol. 33, no. 1, pp. 65-80. https://doi.org/10.1093/humrep/dex326

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

Journal:	*Human Reproduction*
Manuscript ID	HUMREP-17-0337.R2
Manuscript Type:	Meta-Analysis
Date Submitted by the Author:	26-Sep-2017
Complete List of Authors:	Chu, Justin; University of Birmingham, Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research; Birmingham Women's and Children's NHS Foundation Trust Gallos, Ioannis; University of Birmingham, Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research; Birmingham Women's and Children's NHS Foundation Trust Tobias , Aurelio; University of Birmingham, Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research; Spanish Council for Scientific Research, Institute of Environmental Assessment and Water Research Tan, Bee; Heart of England NHS Foundation Trust; University of Warwick, Reproductive Health Eapen, Abey; University of Birmingham, Institue of Metabolism and Systems Research; Birmingham Women's and Children's NHS Foundation Trust Coomarasamy, Arri; University of Birmingham, Institue of Metabolism and Systems Research; Birmingham Women's and Children's NHS Foundation Trust
Keywords:	Vitamin D, IMPLANTATION, Assisted reproductive treatments, In Vitro Fertilisation, ENDOMETRIAL RECEPTIVITY
Subject Section:	Infertility

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

RUNNING TITLE

Vitamin D and assisted reproductive treatment outcome

AUTHORS

Justin Chu[1,2], Ioannis Gallos[1,2*], Aurelio Tobias[1,3], Bee Tan[4,5], Abey Eapen[1,2] and Arri Coomarasamy[1,2]

[1] Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, B15 2TT, UK

[2]Birmingham Women's and Children's NHS Foundation Trust, Mindelsohn Way, Birmingham, B15 2TG, UK

[3]Spanish Council for Scientific Research, Institute of Environmental Assessment and Water Research, Barcelona, Spain

[4]Heartlands Hospital, Heart of England NHS Foundation Trust, Bordesley Green East, Birmingham B9 5SS, UK

[5]Reproductive health, Warwick University, Coventry, CV4 7AL, UK

***CORRESPONDENCE ADDRESS:** Email i.d.gallos@bham.ac.uk, Birmingham Women's Foundation NHS Trust, Edgbaston, B15 2TG

ABSTRACT

Study question: Is serum vitamin D associated with live birth rates in women undergoing assisted reproductive treatment?

Summary answer: Women undergoing assisted reproductive treatment 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 assisted reproductive treatment 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 assisted reproductive treatment outcomes.

Participants/materials, settings, methods: Literature searches were conducted to retrieve studies which reported on the association between vitamin D and assisted reproductive treatment 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 to 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 to 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 to 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 to 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 assisted reproductive treatment. Our results show that vitamin D deficiency and insufficiency could be important conditions to treat in women considering assisted reproductive treatments. A randomised 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 fertilisation / 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 United Kingdom (UK), in 2014, 52,288 women underwent 67,708 in vitro fertilization (IVF) treatment cycles (Human Fertility Embryology Authority 2016). The overall success rate of these assisted reproductive treatments (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 to 52% of women of reproductive age are deficient in vitamin D (Gordon et al. 2004; Sullivan et al. 2005; Tangpricha et al. 2002). 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 less than 50 nmol/L (less than 20ng/mL). A concentration of 50 to 75 nmol/L (21 to 29 ng/mL) is considered insufficient and greater than 75nmol/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 less than 30 nmol/L (less than 12ng/mL), vitamin D insufficency is when serum 25-hydroxy vitamin D_3 concentrations are between 30 nmol/L and 50nmol/L (between 12 and 20ng/mL), and that serum 25-hydroxy vitamin D_3 concentrations greater than 50nmol/L (greater than 20ng/mL) are considered replete (Ross et al. 2011). There is 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. 2014; Ozkan et al. 2010; Rudick et al. 2012; 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 summarise the available evidence.

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, *in vitro* fertilization, intracytoplasmic sperm injection, assisted reproductive techniques 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 assisted reproductive treatments 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 confidence intervals 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 confidence intervals (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).

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 fulfil 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; six were retrospective and five 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 (less than 50nmol/L deficient, 50-75nmol/L insufficient, and greater than 75nmolL 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 Figure 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-analysed prevalence for vitamin D deficiency, insufficiency and replete were 34.6% (95% CI 32.0 to 37.4), 45.3% (95% CI 42.4 to 48.5) and 25.7% (95% CI 23.4 to 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 to 1.65). The meta-analysis had low statistical heterogeneity with an I^2 value of 5.0% (p=0.39).

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 to 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 to 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 less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/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 50nmol/L were more likely to achieve a clinical pregnancy when compared to women with a vitamin D concentration of below 50nmol/L with an odds ratio of 1.38 (1,04 to 1.83) (Supplementary Figure 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 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 to 1.93]). The I^2 value for this meta-analysis was 56.0% suggesting a moderate level of statistical heterogeneity (p=0.02).

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 to 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 to 1.54). There was a low level of statistical heterogeneity denoted by an I^2 value of 0.0% (p=0.76).

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 generalisability of the findings to wider infertility populations.

Ideally, when meta-analysing cohort studies, the adjusted odds ratios (where provided) should be meta-analysed. 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 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 confidence intervals (Schünemann et al. 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.

Acknowledgments

The authors would like to acknowledge Derick Yates (Birmingham Women's and Children's NHS Foundation Trust) who helped design the search strategy for the systematic review and meta-analysis.

Authors' roles

JC and AC were responsible for defining the research question. JC designed the strategy for literature search. JC and BT assessed eligibility of studies for inclusion to the systematic review. Statistical analyses were performed by AT and IDG. AE assisted in the design of the systematic review search strategy and in manuscript preparation. JC 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.

Funding

No external funding was either sought or obtained for this study.

Conflicts of interest

None to declare

References

Aghajafari, F. et al., 2013. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. *BMJ*, 346, p.f1169. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23533188\nhttp://www.bmj.com/content/bmj/346/bmj.f1169.full.pdf.

Aleyasin, A. et al., 2011. Predictive value of the level of vitamin D in follicular fluid on the outcome of assisted reproductive technology. *Eur J Obstet Gynecol Reprod Biol*, 159(1), pp.132–137.

Anagnostis, P., Karras, S. & Goulis, D.G., 2013. Vitamin D in human reproduction: A narrative review. *International Journal of Clinical Practice*, 67(3), pp.225–235.

Anifandis, G.M. et al., 2010. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. *Reproductive biology and endocrinology : RB&E*, 8, p.91.

Baker, A.M. et al., 2010. A Nested Case-Control Study of Midgestation Vitamin D Deficiency and Risk of Severe Preeclampsia. *The Journal of Clinical Endocrinology & Metabolism*, 95(11), pp.5105–5109. Available at: http://press.endocrine.org/doi/abs/10.1210/jc.2010-0996.

Bodnar, L.M. et al., 2007. Maternal vitamin D deficiency increases the risk of preeclampsia. *The Journal of clinical endocrinology and metabolism*, 92(9), pp.3517–22. Available at: http://press.endocrine.org/doi/full/10.1210/jc.2007-0718.

Busso, C.E. et al., 2006. Implantation in IVF. *International Surgery*, 91(5 SUPPL.).

Conde-Agudelo, A. et al., 2013. Novel biomarkers for predicting intrauterine growth restriction: A systematic review and meta-analysis. *BJOG: An International Journal of Obstetrics and Gynaecology*, 120(6), pp.681–694.

Daftary, G.S. & Taylor, H.S., 2006. Endocrine regulation of HOX genes. *Endocrine Reviews*, 27(4), pp.331–355.

De-Regil, L.M. et al., 2012. Vitamin D supplementation for women during pregnancy. *Cochrane database of systematic reviews (Online)*, 2(2), p.CD008873. Available at:

http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008873.pub2/pdf/standard\nhttp://www.ncbi.nlm.nih.gov/pubmed/22336854.

Dhillon, R.K. et al., 2016. Predicting the chance of live birth for women undergoing IVF: a novel pretreatment counselling tool. *Human Reproduction*, 31(1), pp.84–92. Available at: http://humrep.oxfordjournals.org/lookup/doi/10.1093/humrep/dev268.

Evans, K.N. et al., 2004. Vitamin D and placental-decidual function. *Journal of the Society for Gynecologic Investigation*, 11(5), pp.263–271.

Fabris, A. et al., 2014. Impact of circulating levels of total and bioavailable serum vitamin D on pregnancy rate in egg donation recipients. *Fertility and Sterility*, 102(6), pp.1608–1612.

Farzadi, L., Khayatzadeh Bidgoli, H. & Ghojazadeh, M., 2015. Correlation between follicular fluid 25-OH vitamin D and assisted reproductive outcomes. *Iranian Journal of Reproductive Medicine*, 13(6), pp.361–366.

Firouzabadi, R.D. et al., 2014. Value of follicular fluid vitamin D in predicting the pregnancy rate in an IVF program. *Archives of Gynecology and Obstetrics*, 289(1), pp.201–206.

Franasiak, J.M. et al., 2015. Vitamin D levels do not affect IVF outcomes following the transfer of euploid blastocysts. *American Journal of Obstetrics and Gynecology*, 212(3), pp.315.e1–315.e6.

Garbedian, K. et al., 2013. Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization. *CMAJ Open*, 1(2), pp.E77–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25077107.

Gordon, C.M. et al., 2004. Prevalence of vitamin D deficiency among healthy adolescents. *Archives of pediatrics & adolescent medicine*, 158(6), pp.531–537.

Grady, R. et al., 2012. Elective single embryo transfer and perinatal outcomes: A systematic review and meta-analysis. *Fertility and Sterility*, 97(2), pp.324–331.

Halloran, B.P. & DeLuca, H.F., 1980. Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. *J Nutr*, 110(8), pp.1573–1580.

Harbord, R.M., Egger, M. & Sterne, J.., 2006. A modified test for small study effects in meta-analyses

of controlled trials with binary endpoints. *Stat. Med*, 25, pp.3443–3457.

Heaney, R.P., 2008. Vitamin D in health and disease. *Clinical journal of the American Society of Nephrology : CJASN*, 3(5), pp.1535–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18525006.

Holick, M.F. et al., 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of clinical endocrinology and metabolism*, 96(7), pp.1911–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21646368.

Holick, M.F., 2007. Vitamin D deficiency. *The New England journal of medicine*, 357(3), pp.266–281. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17634462.

Human Fertility Embryology Authority, 2016. *Fertility Treatment 2014*,

Ingles, S.A., 2007. Can Diet and/or Sunlight Modify the Relationship between Vitamin D Receptor Polymorphisms and Prostate Cancer Risk? *Nutrition Reviews*, 65(SUPPL.2).

John, E.M. et al., 2007. Sun exposure, vitamin D receptor gene polymorphisms, and breast cancer risk in a multiethnic population. *American Journal of Epidemiology*, 166(12), pp.1409–1419.

Khalessi, N. et al., 2015. The Relationship between Maternal Vitamin D Deficiency and Low Birth Weight Neonates. *Journal of family & reproductive health*, 9(3), pp.113–117. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4662754&tool=pmcentrez&rendertype=abstract\nhttp://www.ncbi.nlm.nih.gov/pubmed/26622309\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4662754.

Kinuta, K. et al., 2000. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology*, 141(4), pp.1317–1324.

Lerchbaum, E. & Rabe, T., 2014. Vitamin D and female fertility. *Current opinion in obstetrics & gynecology*, 26(3), pp.145–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24717915.

Liberati, A. et al., 2009. Annals of Internal Medicine Academia and Clinic The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions : *Annals of Internal Medicine*, 151(4), pp.W65–W94.

Macklon, N.S., Geraedts, J.P.M. & Fauser, B.C.J.M., 2002. Conception to ongoing pregnancy: The "black box" of early pregnancy loss. *Human Reproduction Update*, 8(4), pp.333–343.

Moher, D. et al., 2009. Reprint--preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Physical therapy*, 89(9), pp.873–880.

Moon, R.J., Harvey, N.C. & Cooper, C., 2015. ENDOCRINOLOGY IN PREGNANCY: Influence of maternal vitamin D status on obstetric outcomes and the fetal skeleton. *European Journal of Endocrinology*, 173(2), pp.R69–R83.

National Institute for Health and Care Excellence, N., 2013. Fertility: assessment and treatment for people with fertility problems. *NICE CLincal Guidelines*, (May), p.274–. Available at: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Fertility+:+assessment+and+treatment+for+people+with+fertility+problems#0\nhttp://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Fertility:+assessment+and+treatment+for+people+with+fert.

Neville, G. et al., 2016. Vitamin D status and fertility outcomes during winter among couples undergoing in vitro fertilization/intracytoplasmic sperm injection. *Int J Gynaecol Obstet.*, 135(2), pp.172–176.

Ozkan, S. et al., 2010. Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertility and Sterility*, 94(4), pp.1314–1319.

Paffoni, A. et al., 2014. Vitamin D deficiency and infertility: Insights from in vitro fertilization cycles. *Journal of Clinical Endocrinology and Metabolism*, 99(11), pp.E2372–E2376.

Panda, D.K. et al., 2001. Targeted ablation of the 25-hydroxyvitamin D 1alpha -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *Proceedings of the National Academy of Sciences of the United States of America*, 98(13), pp.7498–7503.

Robinson, C.J. et al., 2011. Maternal vitamin D and fetal growth in early-onset severe preeclampsia. *American Journal of Obstetrics and Gynecology*, 204(6).

Rojansky, N., Brzezinski, A. & Schenker, J.G., 1992. Seasonality in human reproduction: an update. *Human Reproduction*, 7(6), pp.735–745.

503 Ross, A., Manson, J. & Abrams, S., 2011. The 2011 Report on Dietary Reference Intakes for Calcium
504 and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. *The Journal of*
505 *Clinical Endocrinology and Metabolism*, 96(1), pp.53–58.
506 Rudick, B. et al., 2012. Characterizing the influence of vitamin D levels on IVF outcomes. *Human*
507 *Reproduction*, 27(11), pp.3321–3327.
508 Rudick, B. et al., 2010. Characterizing the role of vitamin D levels on IVF outcomes: stimulation,
509 embryo, or endometrium? *Fertility and Sterility*, 94(4), p.S72. Available at:
510 http://linkinghub.elsevier.com/retrieve/pii/S0015028210013853.
511 Rudick, B.J. et al., 2014. Influence of vitamin D levels on in vitro fertilization outcomes in donor-
512 recipient cycles. *Fertility and Sterility*, 101(2), pp.447–452.
513 Schünemann, H. et al., 2011. Cochrane Handbook for Systematic Reviews of Interventions Version
514 5.1.0. In *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0*.
515 Stephanou, A., Ross, R. & Handwerger, S., 1994. Regulation of Human Placental Lactogen Expression
516 by 1,25-Dihydroxyvitamin D3. *Endocrinology*, 135(6), pp.2651–2656.
517 Sullivan, S.S. et al., 2005. Adolescent girls in maine are at risk for vitamin D insufficiency. *Journal of*
518 *the American Dietetic Association*, 105(6), pp.971–974.
519 Tangpricha, V. et al., 2002. Vitamin D insufficiency among free living healthy young adults. *American*
520 *Journal of Medicine*, 112(8), pp.659–662.
521 Wang, O. et al., 2012. Association between vitamin D insufficiency and the risk for gestational
522 diabetes mellitus in pregnant Chinese women. *Biomedical and environmental sciences : BES*,
523 25(4), pp.399–406. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23026519.
524 Wei, S.Q., 2014. Vitamin D and pregnancy outcomes. *Current opinion in obstetrics & gynecology*,
525 26(6), pp.438–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25310531.
526 Wells, G. et al., 2011. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised
527 studies in meta-analysis. 2011. , p.http://www.ohri.ca/programs/clinical_epidemiolog.
528 Yoshizawa, T. et al., 1997. Mice lacking the vitamin D receptor exhibit impaired bone formation,

529 uterine hypoplasia and growth retardation after weaning. *Nature genetics*, 16(4), pp.391–396.
530 Available at:
531 http://www.ncbi.nlm.nih.gov/pubmed/9241280\nhttp://www.nature.com/ng/journal/v16/n4/
532 abs/ng0897-391.html.
533 Zhang, M.X. et al., 2015. Vitamin D deficiency increases the risk of gestational diabetes mellitus: A
534 meta-analysis of observational studies. *Nutrients*, 7(10), pp.8366–8375. Available at:
535 http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed13&AN=2
536 015431828\nhttp://sfx.ucl.ac.uk/sfx_local?sid=OVID:embase&id=pmid:&id=doi:10.3390/nu710
537 5398&issn=2072-6643&isbn=&volume=7&issue=10&spage=8366&pages=8366-
538 8375&date=2015&title.
539
540
541

Figure Legends

Figure 1. PRISMA flow diagram for study selection.

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)

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.

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.

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.

Supplementary Figure S1. Vitamin D and *in vitro* fertilisation treatment clinical pregnancy outcomes publication bias funnel plot. The funnel plot to test for asymmetry showed no substantial evidence of publication bias.

Supplementary Figure S2. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations implementing Institute of Medicine cut-offs. Data could be extracted from nine of the included studies to compare the chances of clinical pregnancy by using the Institute of Medicine definitions of vitamin D status (vitamin D concentrations of less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/L considered replete). Meta-analysis of the data from these nine studies 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 according to Institute of Medicine vitamin D cut-offs.

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

RUNNING TITLE

Vitamin D and assisted reproductive treatment outcome

AUTHORS

Justin Chu[1,2], Ioannis Gallos[1,2*], Aurelio Tobias[1,3], Bee Tan[4,5], Abey Eapen[1,2] and Arri Coomarasamy[1,2]

[1] Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, B15 2TT, UK

[2]Birmingham Women's and Children's NHS Foundation Trust, Mindelsohn Way, Birmingham, B15 2TG, UK

[3]Spanish Council for Scientific Research, Institute of Environmental Assessment and Water Research, Barcelona, Spain

[4]Heartlands Hospital, Heart of England NHS Foundation Trust, Bordesley Green East, Birmingham B9 5SS, UK

[5]Reproductive health, Warwick University, Coventry, CV4 7AL, UK

***CORRESPONDENCE ADDRESS:** Email i.d.gallos@bham.ac.uk, Birmingham Women's Foundation NHS Trust, Edgbaston, B15 2TG

ABSTRACT

Study question: Is serum vitamin D associated with live birth rates in women undergoing assisted reproductive treatment?

Summary answer: Women undergoing assisted reproductive treatment 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 assisted reproductive treatment 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 assisted reproductive treatment outcomes.

Participants/materials, settings, methods: Literature searches were conducted to retrieve studies which reported on the association between vitamin D and assisted reproductive treatment 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 to 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 to 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 to 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 to 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 assisted reproductive treatment. Our results show that vitamin D deficiency and insufficiency could be important conditions to treat in women considering assisted reproductive treatments. A randomised 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 fertilisation / 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 United Kingdom (UK), in 2014, 52,288 women underwent 67,708 in vitro fertilization (IVF) treatment cycles (Human Fertility Embryology Authority 2016). The overall success rate of these assisted reproductive treatments (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 to 52% of women of reproductive age are deficient in vitamin D (Gordon et al. 2004; Sullivan et al. 2005; Tangpricha et al. 2002). 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 less than 50 nmol/L (less than 20ng/mL). A concentration of 50 to 75 nmol/L (21 to 29 ng/mL) is considered insufficient and greater than 75nmol/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 less than 30 nmol/L (less than 12ng/mL), vitamin D insufficency is when serum 25-hydroxy vitamin D_3 concentrations are between 30 nmol/L and 50nmol/L (between 12 and 20ng/mL), and that serum 25-hydroxy vitamin D_3 concentrations greater than 50nmol/L (greater than 20ng/mL) are considered replete (Ross et al. 2011). There is 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. 2014; Ozkan et al. 2010; Rudick et al. 2012; 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 summarise the available evidence.

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, *in vitro* fertilization, intracytoplasmic sperm injection, assisted reproductive techniques 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 assisted reproductive treatments 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 confidence intervals 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 confidence intervals (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).

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 fulfil 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; six were retrospective and five 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 (less than 50nmol/L deficient, 50-75nmol/L insufficient, and greater than 75nmolL 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 Figure 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-analysed prevalence for vitamin D deficiency, insufficiency and replete were 34.6% (95% CI 32.0 to 37.4), 45.3% (95% CI 42.4 to 48.5) and 25.7% (95% CI 23.4 to 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 to 1.65). The meta-analysis had low statistical heterogeneity with an I^2 value of 5.0% (p=0.39).

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 to 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 to 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 less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/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 50nmol/L were more likely to achieve a clinical pregnancy when compared to women with a vitamin D concentration of below 50nmol/L with an odds ratio of 1.38 (1,04 to 1.83) (Supplementary Figure 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 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 to 1.93]). The I^2 value for this meta-analysis was 56.0% suggesting a moderate level of statistical heterogeneity (p=0.02).

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 to 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 to 1.54). There was a low level of statistical heterogeneity denoted by an I^2 value of 0.0% (p=0.76).

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 generalisability of the findings to wider infertility populations.

Ideally, when meta-analysing cohort studies, the adjusted odds ratios (where provided) should be meta-analysed. 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 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~~does~~ suggest ~~show~~ ~~a trend towards~~ 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 confidence intervals (Schünemann et al. 2011). Removal of these two studies from the overall analysis did not alter the overall association between vitamin D concentration and clinical pregnancy.

Comment [JC1]: I have just deleted 3 words here. Deleting the whole sentence would lose the message that is being conveyed.

Comment [RC2]: The use of the term trend is misleading as trend analysis was not performed and interpretation may be bias to an expected outcome. I would recommend deleting the sentence.

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

Comment [RC3]: Such as a diet deficiency in general? If so would it be beneficial to diagnose for other micro-nutrient deficiencies?

Comment [JC4]: Vitamin D has been suggested to be a surrogate marker for general well-being. I have added the reference here. The same reference has been used previously in the manuscript so there is no need to change the reference list.

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.

Acknowledgments

The authors would like to acknowledge Derick Yates (Birmingham Women's and Children's NHS Foundation Trust) who helped design the search strategy for the systematic review and meta-analysis.

Authors' roles

JC and AC were responsible for defining the research question. JC designed the strategy for literature search. JC and BT assessed eligibility of studies for inclusion to the systematic review. Statistical analyses were performed by AT and IDG. AE assisted in the design of the systematic review search strategy and in manuscript preparation. JC 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.

Comment [RC5]: Please include the details of the contributions from all authors; AE is missing.

Comment [JC6]: AE author contribution added

Funding

No external funding was either sought or obtained for this study.

Conflicts of interest

None to declare

References

Aghajafari, F. et al., 2013. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. *BMJ*, 346, p.f1169. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23533188\nhttp://www.bmj.com/content/bmj/346/bmj.f1169.full.pdf.

Aleyasin, A. et al., 2011. Predictive value of the level of vitamin D in follicular fluid on the outcome of assisted reproductive technology. *Eur J Obstet Gynecol Reprod Biol*, 159(1), pp.132–137.

Anagnostis, P., Karras, S. & Goulis, D.G., 2013. Vitamin D in human reproduction: A narrative review. *International Journal of Clinical Practice*, 67(3), pp.225–235.

Anifandis, G.M. et al., 2010. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. *Reproductive biology and endocrinology : RB&E*, 8, p.91.

Baker, A.M. et al., 2010. A Nested Case-Control Study of Midgestation Vitamin D Deficiency and Risk of Severe Preeclampsia. *The Journal of Clinical Endocrinology & Metabolism*, 95(11), pp.5105–5109. Available at: http://press.endocrine.org/doi/abs/10.1210/jc.2010-0996.

Bodnar, L.M. et al., 2007. Maternal vitamin D deficiency increases the risk of preeclampsia. *The Journal of clinical endocrinology and metabolism*, 92(9), pp.3517–22. Available at: http://press.endocrine.org/doi/full/10.1210/jc.2007-0718.

Busso, C.E. et al., 2006. Implantation in IVF. *International Surgery*, 91(5 SUPPL.).

Conde-Agudelo, A. et al., 2013. Novel biomarkers for predicting intrauterine growth restriction: A systematic review and meta-analysis. *BJOG: An International Journal of Obstetrics and Gynaecology*, 120(6), pp.681–694.

Daftary, G.S. & Taylor, H.S., 2006. Endocrine regulation of HOX genes. *Endocrine Reviews*, 27(4), pp.331–355.

De-Regil, L.M. et al., 2012. Vitamin D supplementation for women during pregnancy. *Cochrane database of systematic reviews (Online)*, 2(2), p.CD008873. Available at:

http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008873.pub2/pdf/standard\nhttp://www.ncbi.nlm.nih.gov/pubmed/22336854.

Dhillon, R.K. et al., 2016. Predicting the chance of live birth for women undergoing IVF: a novel pretreatment counselling tool. *Human Reproduction*, 31(1), pp.84–92. Available at: http://humrep.oxfordjournals.org/lookup/doi/10.1093/humrep/dev268.

Evans, K.N. et al., 2004. Vitamin D and placental-decidual function. *Journal of the Society for Gynecologic Investigation*, 11(5), pp.263–271.

Fabris, A. et al., 2014. Impact of circulating levels of total and bioavailable serum vitamin D on pregnancy rate in egg donation recipients. *Fertility and Sterility*, 102(6), pp.1608–1612.

Farzadi, L., Khayatzadeh Bidgoli, H. & Ghojazadeh, M., 2015. Correlation between follicular fluid 25-OH vitamin D and assisted reproductive outcomes. *Iranian Journal of Reproductive Medicine*, 13(6), pp.361–366.

Firouzabadi, R.D. et al., 2014. Value of follicular fluid vitamin D in predicting the pregnancy rate in an IVF program. *Archives of Gynecology and Obstetrics*, 289(1), pp.201–206.

Franasiak, J.M. et al., 2015. Vitamin D levels do not affect IVF outcomes following the transfer of euploid blastocysts. *American Journal of Obstetrics and Gynecology*, 212(3), pp.315.e1–315.e6.

Garbedian, K. et al., 2013. Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization. *CMAJ Open*, 1(2), pp.E77–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25077107.

Gordon, C.M. et al., 2004. Prevalence of vitamin D deficiency among healthy adolescents. *Archives of pediatrics & adolescent medicine*, 158(6), pp.531–537.

Grady, R. et al., 2012. Elective single embryo transfer and perinatal outcomes: A systematic review and meta-analysis. *Fertility and Sterility*, 97(2), pp.324–331.

Halloran, B.P. & DeLuca, H.F., 1980. Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. *J Nutr*, 110(8), pp.1573–1580.

Harbord, R.M., Egger, M. & Sterne, J.., 2006. A modified test for small study effects in meta-analyses

of controlled trials with binary endpoints. *Stat. Med*, 25, pp.3443–3457.

Heaney, R.P., 2008. Vitamin D in health and disease. *Clinical journal of the American Society of Nephrology : CJASN*, 3(5), pp.1535–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18525006.

Holick, M.F. et al., 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of clinical endocrinology and metabolism*, 96(7), pp.1911–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21646368.

Holick, M.F., 2007. Vitamin D deficiency. *The New England journal of medicine*, 357(3), pp.266–281. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17634462.

Human Fertility Embryology Authority, 2016. *Fertility Treatment 2014*,

Ingles, S.A., 2007. Can Diet and/or Sunlight Modify the Relationship between Vitamin D Receptor Polymorphisms and Prostate Cancer Risk? *Nutrition Reviews*, 65(SUPPL.2).

John, E.M. et al., 2007. Sun exposure, vitamin D receptor gene polymorphisms, and breast cancer risk in a multiethnic population. *American Journal of Epidemiology*, 166(12), pp.1409–1419.

Khalessi, N. et al., 2015. The Relationship between Maternal Vitamin D Deficiency and Low Birth Weight Neonates. *Journal of family & reproductive health*, 9(3), pp.113–117. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4662754&tool=pmcentrez&rendertype=abstract\nhttp://www.ncbi.nlm.nih.gov/pubmed/26622309\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4662754.

Kinuta, K. et al., 2000. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology*, 141(4), pp.1317–1324.

Lerchbaum, E. & Rabe, T., 2014. Vitamin D and female fertility. *Current opinion in obstetrics & gynecology*, 26(3), pp.145–50. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24717915.

Liberati, A. et al., 2009. Annals of Internal Medicine Academia and Clinic The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions : *Annals of Internal Medicine*, 151(4), pp.W65–W94.

Macklon, N.S., Geraedts, J.P.M. & Fauser, B.C.J.M., 2002. Conception to ongoing pregnancy: The "black box" of early pregnancy loss. *Human Reproduction Update*, 8(4), pp.333–343.

Moher, D. et al., 2009. Reprint--preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Physical therapy*, 89(9), pp.873–880.

Moon, R.J., Harvey, N.C. & Cooper, C., 2015. ENDOCRINOLOGY IN PREGNANCY: Influence of maternal vitamin D status on obstetric outcomes and the fetal skeleton. *European Journal of Endocrinology*, 173(2), pp.R69–R83.

National Institute for Health and Care Excellence, N., 2013. Fertility: assessment and treatment for people with fertility problems. *NICE CLincal Guidelines*, (May), p.274–. Available at: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Fertility+:+assessment+and+treatment+for+people+with+fertility+problems#0\nhttp://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Fertility:+assessment+and+treatment+for+people+with+fert.

Neville, G. et al., 2016. Vitamin D status and fertility outcomes during winter among couples undergoing in vitro fertilization/intracytoplasmic sperm injection. *Int J Gynaecol Obstet.*, 135(2), pp.172–176.

Ozkan, S. et al., 2010. Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertility and Sterility*, 94(4), pp.1314–1319.

Paffoni, A. et al., 2014. Vitamin D deficiency and infertility: Insights from in vitro fertilization cycles. *Journal of Clinical Endocrinology and Metabolism*, 99(11), pp.E2372–E2376.

Panda, D.K. et al., 2001. Targeted ablation of the 25-hydroxyvitamin D 1alpha -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *Proceedings of the National Academy of Sciences of the United States of America*, 98(13), pp.7498–7503.

Robinson, C.J. et al., 2011. Maternal vitamin D and fetal growth in early-onset severe preeclampsia. *American Journal of Obstetrics and Gynecology*, 204(6).

Rojansky, N., Brzezinski, A. & Schenker, J.G., 1992. Seasonality in human reproduction: an update. *Human Reproduction*, 7(6), pp.735–745.

503 Ross, A., Manson, J. & Abrams, S., 2011. The 2011 Report on Dietary Reference Intakes for Calcium
504 and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. *The Journal of*
505 *Clinical Endocrinology and Metabolism*, 96(1), pp.53–58.
506 Rudick, B. et al., 2012. Characterizing the influence of vitamin D levels on IVF outcomes. *Human*
507 *Reproduction*, 27(11), pp.3321–3327.
508 Rudick, B. et al., 2010. Characterizing the role of vitamin D levels on IVF outcomes: stimulation,
509 embryo, or endometrium? *Fertility and Sterility*, 94(4), p.S72. Available at:
510 http://linkinghub.elsevier.com/retrieve/pii/S0015028210013853.
511 Rudick, B.J. et al., 2014. Influence of vitamin D levels on in vitro fertilization outcomes in donor-
512 recipient cycles. *Fertility and Sterility*, 101(2), pp.447–452.
513 Schünemann, H. et al., 2011. Cochrane Handbook for Systematic Reviews of Interventions Version
514 5.1.0. In *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0*.
515 Stephanou, A., Ross, R. & Handwerger, S., 1994. Regulation of Human Placental Lactogen Expression
516 by 1,25-Dihydroxyvitamin D3. *Endocrinology*, 135(6), pp.2651–2656.
517 Sullivan, S.S. et al., 2005. Adolescent girls in maine are at risk for vitamin D insufficiency. *Journal of*
518 *the American Dietetic Association*, 105(6), pp.971–974.
519 Tangpricha, V. et al., 2002. Vitamin D insufficiency among free living healthy young adults. *American*
520 *Journal of Medicine*, 112(8), pp.659–662.
521 Wang, O. et al., 2012. Association between vitamin D insufficiency and the risk for gestational
522 diabetes mellitus in pregnant Chinese women. *Biomedical and environmental sciences : BES*,
523 25(4), pp.399–406. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23026519.
524 Wei, S.Q., 2014. Vitamin D and pregnancy outcomes. *Current opinion in obstetrics & gynecology*,
525 26(6), pp.438–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25310531.
526 Wells, G. et al., 2011. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised
527 studies in meta-analysis. 2011. , p.http://www.ohri.ca/programs/clinical_epidemiolog.
528 Yoshizawa, T. et al., 1997. Mice lacking the vitamin D receptor exhibit impaired bone formation,

529 uterine hypoplasia and growth retardation after weaning. *Nature genetics*, 16(4), pp.391–396.
530 Available at:
531 http://www.ncbi.nlm.nih.gov/pubmed/9241280\nhttp://www.nature.com/ng/journal/v16/n4/
532 abs/ng0897-391.html.
533 Zhang, M.X. et al., 2015. Vitamin D deficiency increases the risk of gestational diabetes mellitus: A
534 meta-analysis of observational studies. *Nutrients*, 7(10), pp.8366–8375. Available at:
535 http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed13&AN=2
536 015431828\nhttp://sfx.ucl.ac.uk/sfx_local?sid=OVID:embase&id=pmid:&id=doi:10.3390/nu710
537 5398&issn=2072-6643&isbn=&volume=7&issue=10&spage=8366&pages=8366-
538 8375&date=2015&title.
539
540
541

Figure Legends

Figure 1. PRISMA flow diagram for study selection.

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)

Comment [RC7]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

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.

Comment [RC8]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

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.

Comment [RC9]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

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.

Comment [RC10]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

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.

Comment [RC11]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

Supplementary Figure S1. Vitamin D and *in vitro* fertilisation treatment clinical pregnancy outcomes publication bias funnel plot. The funnel plot to test for asymmetry showed no substantial evidence of publication bias.

Comment [RC12]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

Supplementary Figure S2. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations implementing Institute of Medicine cut-offs. Data could be extracted from nine of the included studies to compare the chances of clinical pregnancy by using the Institute of Medicine definitions of vitamin D status (vitamin D concentrations of less than 50nmol/L considered as deficient or insufficient and vitamin D concentrations of more than 50nmol/L considered replete). Meta-analysis of the data from these nine studies 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 according to Institute of Medicine vitamin D cut-offs.

Comment [RC13]: Please give a brief description interpreting the plot, so the figure stands alone without needing to refer back to the main text.

Figure 1. PRISMA flow diagram for study selection.

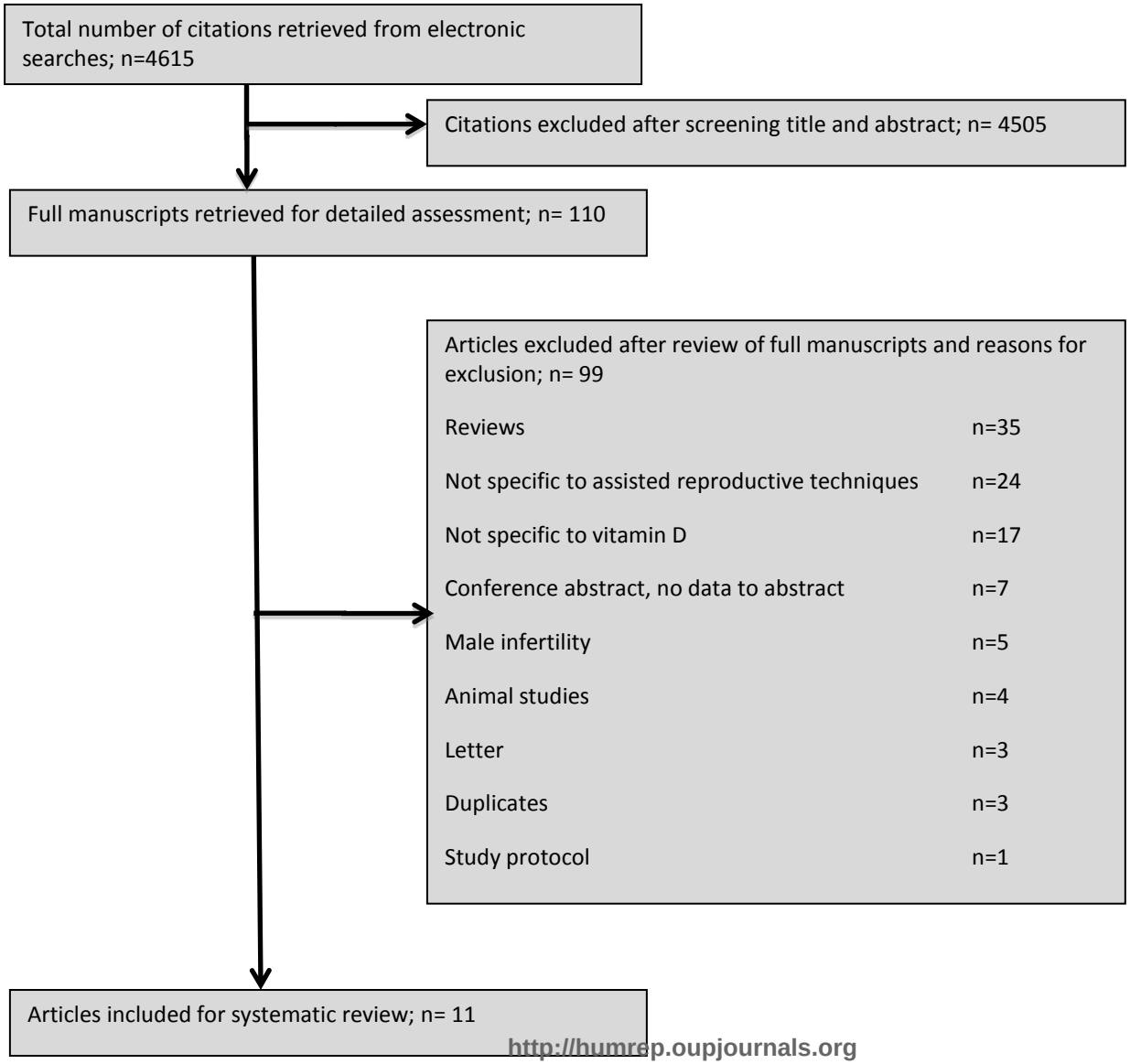


Figure 2. Meta-analysis of studies reporting live birth by vitamin D concentrations

Study	Vit D replete Events	Vit D replete Total	Vit D deficiency/ insufficiency Events	Vit D deficiency/ insufficiency Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Fabris 2014	23	41	127	226	12.0%	1.00 [0.51, 1.95]
Franasiak 2015	60	96	264	421	25.7%	0.99 [0.63, 1.57]
Fru 2014	25	58	20	65	7.5%	1.70 [0.81, 3.57]
Paffoni 2014	19	64	57	271	10.7%	1.59 [0.86, 2.92]
Polyzos 2014	61	129	139	368	26.6%	1.48 [0.99, 2.22]
Rudick 2012	26	79	33	109	13.0%	1.13 [0.61, 2.10]
Rudick 2014	20	35	21	64	4.5%	2.73 [1.17, 6.38]
Total (95% CI)		**502**		**1524**	**100.0%**	**1.33 [1.08, 1.65]**
Total events	234		661			

Heterogeneity: Chi² = 6.33, df = 6 (P = 0.39); I² = 5%

Test for overall effect: Z = 2.62 (P = 0.009)

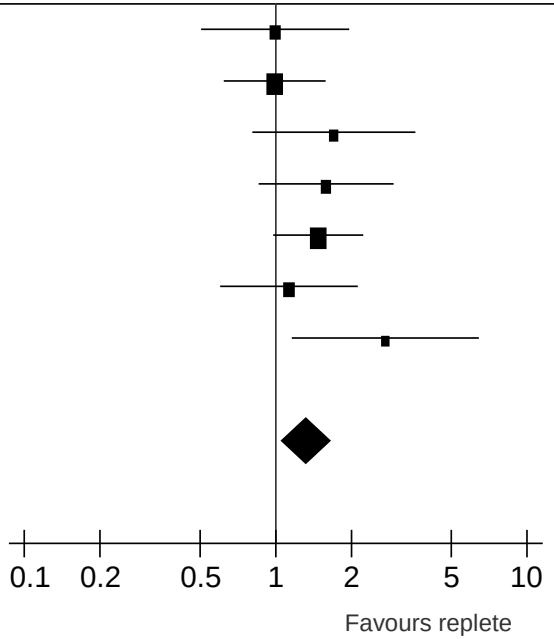


Figure 3. Meta-analysis of studies reporting biochemical pregnancy by vitamin D concentrations

Study	Vit D replete Events	Total	Vit D deficiency/ insufficiency Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Fabris 2014	25	41	145	226	17.1%	0.87 [0.44, 1.73]
Franasiak 2015	74	96	325	421	27.2%	0.99 [0.59, 1.68]
Ozkan 2008	8	31	7	53	3.8%	2.29 [0.74, 7.08]
Paffoni 2014	25	64	70	271	16.1%	1.84 [1.04, 3.26]
Polyzos 2014	86	129	210	368	35.8%	1.50 [0.99, 2.29]
Total (95% CI)		**361**		**1339**	**100.0%**	**1.34 [1.04, 1.73]**
Total events	218		757			

Heterogeneity: $Chi^2 = 5.08$, df = 4 (P = 0.28); $I^2 = 21\%$

Test for overall effect: Z = 2.23 (P = 0.03)

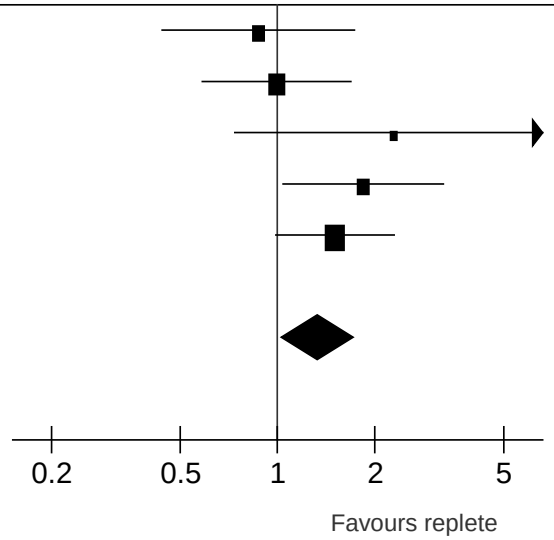


Figure 4. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations

Study or Subgroup	Vit D replete Events	Total	Vit D deficiency/ insufficiency Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Anifanidis 2010	3	21	26	80	4.5%	0.35 [0.09, 1.28]
Fabris 2014	29	41	162	226	9.0%	0.95 [0.46, 1.99]
Firouzabadi 2014	4	16	70	205	5.3%	0.64 [0.20, 2.07]
Franasiak 2014	64	96	295	421	12.2%	0.85 [0.53, 1.37]
Fru 2014	37	58	30	65	9.1%	2.06 [1.00, 4.24]
Garbedian 2013	41	78	66	190	11.4%	2.08 [1.22, 3.56]
Ozkan 2008	15	31	11	53	6.7%	3.58 [1.36, 9.42]
Paffoni 2014	23	64	63	271	10.8%	1.85 [1.03, 3.32]
Polyzos 2014	70	129	168	368	13.1%	1.41 [0.94, 2.11]
Rudick 2012	34	79	43	109	10.7%	1.16 [0.64, 2.09]
Rudick 2014	26	35	25	64	7.2%	4.51 [1.82, 11.19]
Total (95% CI)		**648**		**2052**	**100.0%**	**1.46 [1.05, 2.02]**
Total events	346		959			

Heterogeneity: Tau² = 0.17; Chi² = 25.73, df = 10 (P = 0.004); I² = 61%

Test for overall effect: Z = 2.24 (P = 0.02)

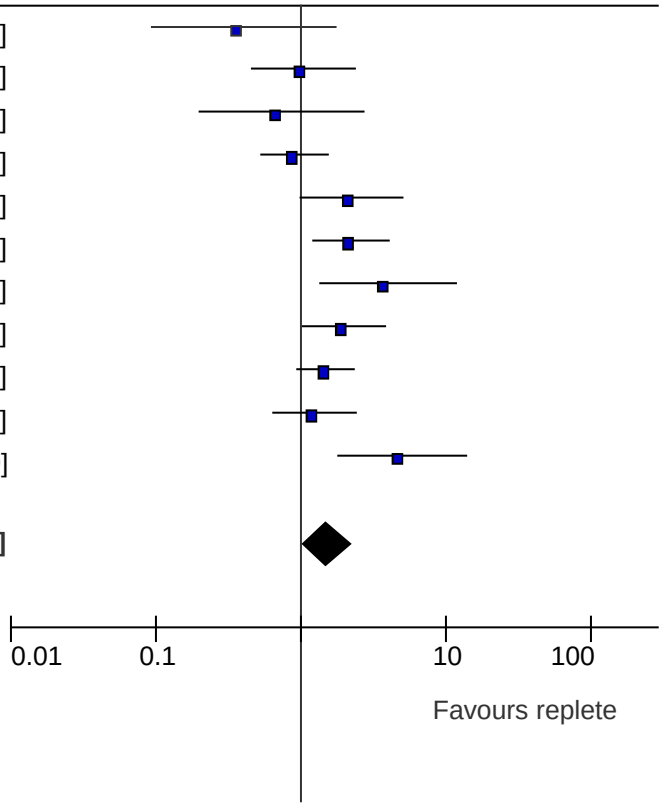


Figure 5. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations according to source of oocyte

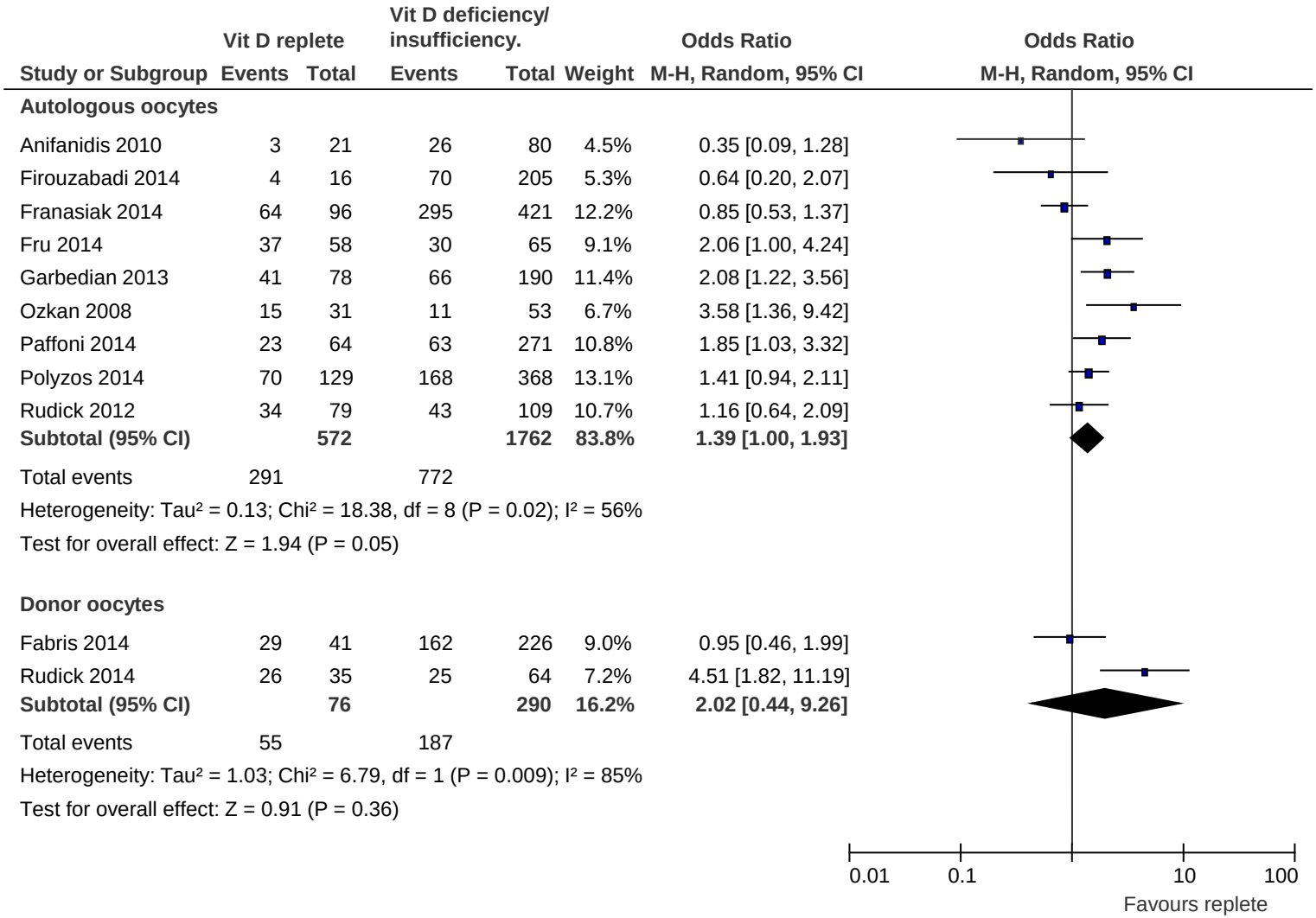


Figure 6. Meta-analysis of studies reporting miscarriage by vitamin D concentrations

Study	Vit D replete Events	Vit D replete Total	Vit D deficiency/ insufficiency Events	Vit D deficiency/ insufficiency Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Fabris 2014	4	41	17	226	6.7%	1.33 [0.42, 4.17]
Franasiak 2015	14	96	61	421	27.5%	1.01 [0.54, 1.89]
Fru 2014	12	37	10	30	10.6%	0.96 [0.34, 2.67]
Polyzos 2014	25	129	69	368	41.1%	1.04 [0.63, 1.73]
Rudick 2012	8	79	10	109	10.7%	1.12 [0.42, 2.97]
Rudick 2014	6	35	4	64	3.3%	3.10 [0.81, 11.86]
Total (95% CI)		**417**		**1218**	**100.0%**	**1.12 [0.81, 1.54]**
Total events	69		171			

Heterogeneity: $Chi^2 = 2.58$, df = 5 (P = 0.76); $I^2 = 0\%$

Test for overall effect: Z = 0.69 (P = 0.49)

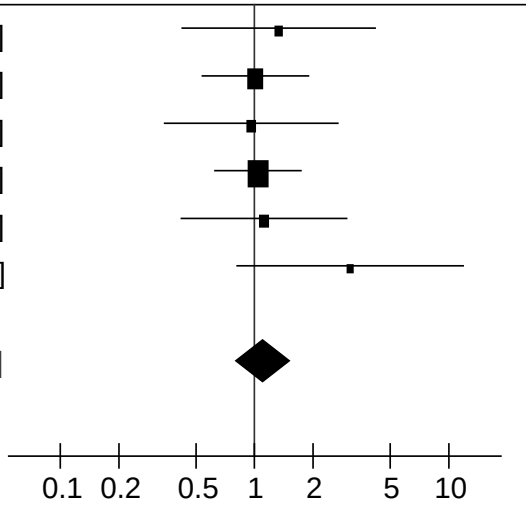


Table I. Characteristics of included studies

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 utilised	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 electrochemilumi nescence immunoassay (ECLIA)	Deficien cy <50nmo l/L Insuffici ency 50-75nmol/ L Replete >75nmo l/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 enzyme-linked immunosorbent assay (ELISA)	Deficien cy <50nmo l/L Insuffici ency 50-75nmol/ L Replete >75nmo l/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
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 enzyme-linked immunosorbent assay (ELISA)	Deficien cy <25nmo l/L Insuffici ency 25-75nmol/ L Replete >75nmo l/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 enzyme-linked immunosorbent assay (ELISA)	Deficiency <50nmol/L Insufficiency 50-75nmol/L Replete >75nmol/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 <50nmol/L Insufficiency 50-75nmol/L Replete >75nmol/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 <75nmol/L Replete >75nmol/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 (>75nmol/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 <50nmol/L Insuffici	Autologous	Clinical pregnancy (intrauterine sac seen on ultrasound scan [no time point defined]) 5/23 deficient group 6/30 insufficient group	Miscarriage Data not provided Live birth	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 (20-30ng/ml) and

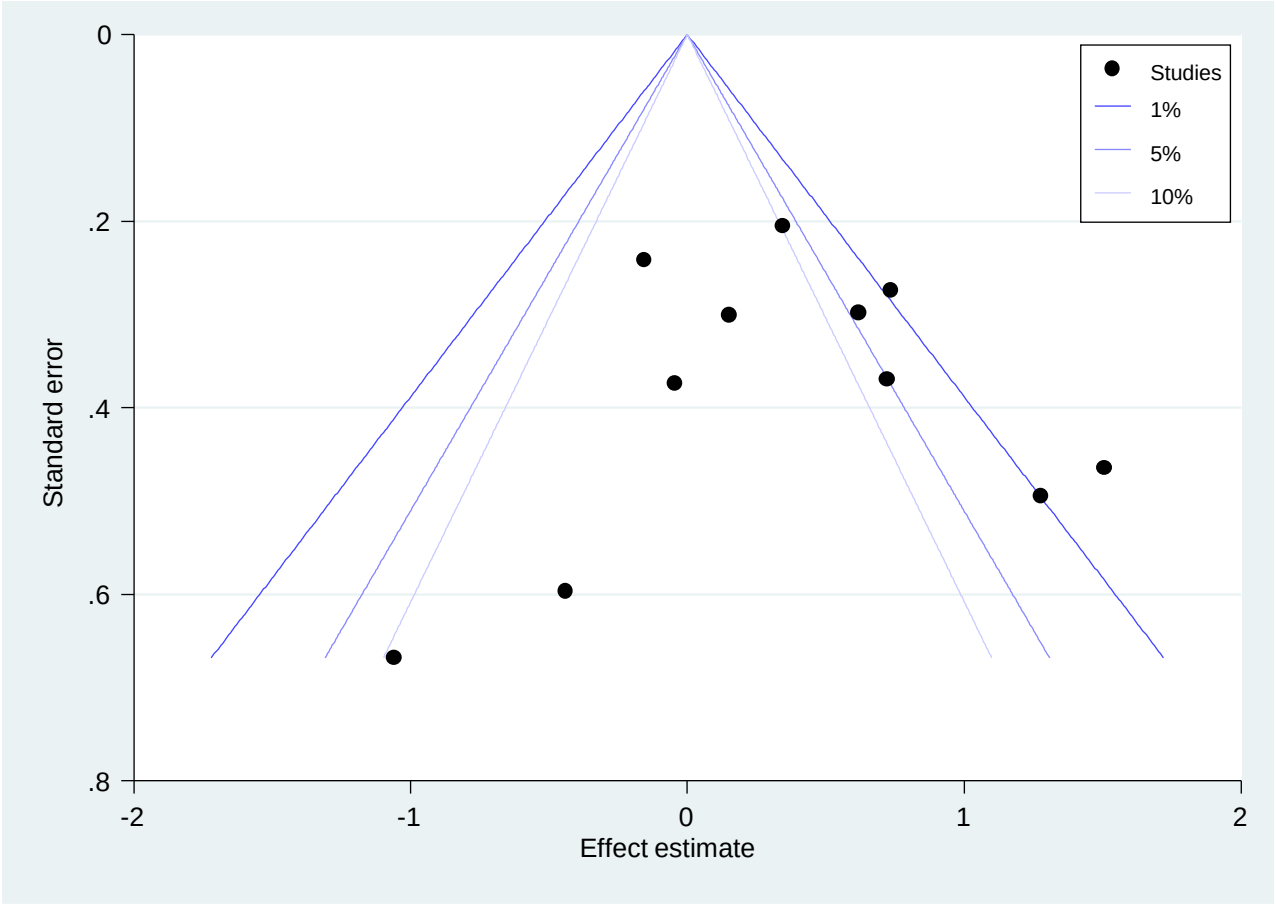
							ency 50-75nmol/L Replete >75nmol/L		15/31 replete group Pregnancy test positive (pregnancy test positive 2 weeks after embryo transfer) 3/23 deficient group 4/30 insufficient group 8/31 replete group	Data not provided		replete concentrations (>30ng/ml) when compared to deficient group - highest in replete 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 electrochemilumi nescence immunoassay (ECLIA)	Deficiency <50nmol/L Insufficiency 50-75nmol/L Replete >75nmol/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 enzyme-linked immunosorbent assay (ELISA)	Deficiency <50nmol/L Insufficiency 50-75nmol/L Replete >75nmol/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 enzyme-linked immunosorbent assay (ELISA)	Deficiency <50nmol/L Insufficiency 50-75nmol/L Replete >75nmol/L	Autologous	Clinical pregnancy (intrauterine sac seen 5 weeks on ultrasound scan after embryo transfer) 14/39 deficient group 29/70 insufficient group 34/79 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) 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	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
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 enzyme-linked immunosorbent assay (ELISA)	Deficiency <50nmol/L Insufficiency 50-75nmol/L Replete >75nmol/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 localised 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

Supplementary Figure S1. Vitamin D and in vitro fertilisation treatment clinical pregnancy outcomes publication bias funnel plot

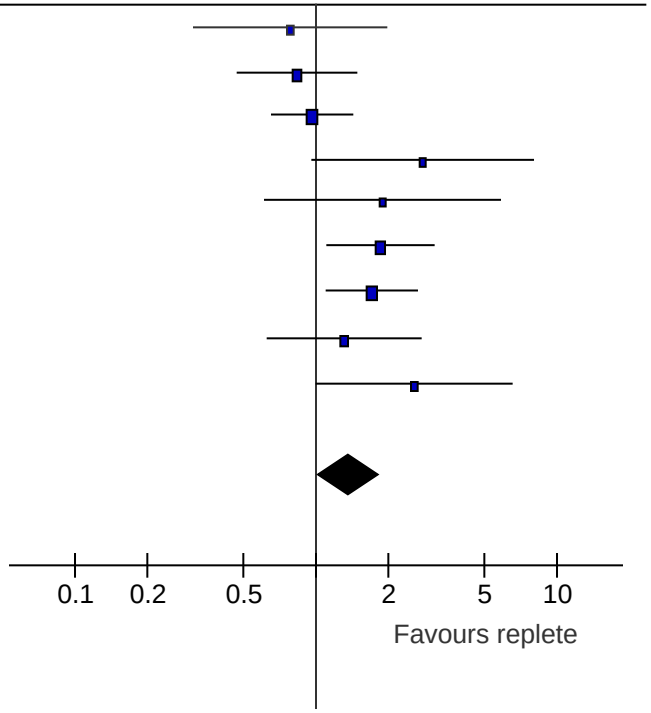


Supplementary Figure S2. Meta-analysis of studies reporting clinical pregnancy by vitamin D concentrations implementing Institute of Medicine cut-offs

Study	Vit D replete Events	Vit D replete Total	Vit D Insufficiency/ deficient Events	Vit D Insufficiency/ deficient Total	Weight	Odds Ratio M-H, Random, 95% CI
Anifanidis 2010	19	70	10	31	7.1%	0.78 [0.31, 1.96]
Fabris 2014	123	175	68	92	13.4%	0.83 [0.47, 1.47]
Franasiak 2014	215	311	144	206	19.1%	0.96 [0.66, 1.41]
Fru 2014	61	105	6	18	5.8%	2.77 [0.97, 7.95]
Ozkan 2008	21	61	5	23	5.2%	1.89 [0.61, 5.81]
Paffoni 2014	56	181	30	154	15.1%	1.85 [1.11, 3.08]
Polyzos 2014	70	129	98	239	17.4%	1.71 [1.11, 2.63]
Rudick 2012	63	149	14	39	9.9%	1.31 [0.63, 2.72]
Rudick 2014	42	73	9	26	7.0%	2.56 [1.01, 6.50]
Total (95% CI)		**1254**		**828**	**100.0%**	**1.38 [1.04, 1.83]**
Total events	670		384			

Heterogeneity: Tau² = 0.07; Chi² = 13.64, df = 8 (P = 0.09); I² = 41%

Test for overall effect: Z = 2.23 (P = 0.03)



Supplementary Table S1

Full electronic search strategy: Vitamin D and Assisted Reproductive Treatment Outcomes

Line	*Database*	*Search term*
1	*Medline*	*"pregnancy".ti,ab*
2	*Medline*	*"in vitro fertilisation".ti,ab*
3	*Medline*	*"intracytoplasmic sperm injection".ti,ab*
4	*Medline*	*"assisted reproductive treatment".ti,ab*
5	*Medline*	*1 OR 2 OR 3 OR 4*
6	*Medline*	*VITAMIN D/*
7	*Medline*	*((vitamin ADJ D)).ti,ab*
8	*Medline*	*((cholecalciferol OR ergocalciferol)).ti,ab*
9	*Medline*	*6 OR 7 OR 8*
10	*Medline*	*5 AND 9*

ti; title, ab; abstract, ADJ; adjacent

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 3 to 4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 5 to 7
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 7
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Not registered
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary File S1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 8 to 9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 7 to 9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Page 9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 9 and supplementary file S2
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 7 to 9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 9 and figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 10 and table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 11, supplementary file S2 and table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 11 to 13, Figures 2 to 6 and Table 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 11 to 13 and Figures 2 to 6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 11 and supplementary file S2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 12 to 13, Figure 5 and supplementary file S3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 13-14

PRISMA 2009 Checklist

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 14 to 17
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Page 18

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.