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# Gestational Vitamin D and Offspring Risk of Multiple Sclerosis: A Systematic Review and Meta-Analysis

Short title: Gestational Vitamin D and Multiple Sclerosis

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## Abstract

**Purpose:** Our objective was to systematically review and meta-analyze studies that assessed the association between gestational vitamin D levels and risk of multiple sclerosis (MS) in offspring.

**Methods:** Embase and Pubmed databases were searched from inception to May 2018. Original, observational studies that investigated both clinically defined MS (in offspring) and vitamin D levels in utero or shortly after birth were included. Two reviewers independently abstracted data and assessed the quality of studies using the Newcastle-Ottawa Quality Assessment Scale. Summary effect estimates and 95% confidence intervals were calculated with random effects models using inverse variance weighting. Determinants of heterogeneity were evaluated.

**Results:** Four case-control studies of moderate to low risk of bias were included. Summary effect estimates of the effect of higher levels of gestational vitamin D on risk of offspring MS demonstrated a significant protective effect in random-effects (OR: 0.63, 95% CI: 0.47, 0.84) models and in a stratified analysis based on study quality. Factors identified as determinants of heterogeneity were the definitions of vitamin D deficiency, the characteristics of study participants, and the quality of the study.

**Conclusions:** Sufficient levels of vitamin D during pregnancy may be protective against offspring's development of multiple sclerosis later in life.


**Suggested Keywords:** "Multiple Sclerosis", "Vitamin D", "Gestation", "Pregnancy", "Meta-Analysis"

**List of abbreviations:** multiple sclerosis (MS)

## Background

Multiple sclerosis (MS) is a neuroinflammatory, demyelinating autoimmune disease of the central nervous system[1]. The disease process associated with MS results in inflammation, damage, and/or destruction of myelin, the fatty component of the membrane (myelin sheath) which surrounds and protects nerve fibers (axons). Thus, the damage produced by this chronic disease results in distorted or interrupted transmission of nerve impulses between the body, spinal cord, and brain[1]. Consequently, this disruption of nerve signaling generates the broad range of symptoms and disabilities associated with MS.

As the most common debilitating, neurological disease in young adults, multiple sclerosis affects roughly 2.3 million individuals worldwide[2, 3]. Furthermore, several studies have indicated that the rate of MS has been increasing throughout the twentieth century[2]. Therefore, multiple sclerosis is a growing public health concern. However, public health endeavors are limited given that the exact etiology of the disease remains unknown. Risk of MS is multifactorial, as genetic and environmental factors have been hypothesized to play a role in risk and development of MS. Genetic studies have revealed the familial aggregation of the disease, with first degree relatives of individuals with MS possessing 10 to 25 times greater risk than the general population[4], with an estimated heritability of 25%[3, 5].

Vitamin D (25-hydroxyvitamin D/25[OH]D) was one of the earliest hypothesized risk factors for MS, largely due to the higher rates of MS observed at increasing latitudes[1]. Though controversial, studies regarding time (month) of birth discovered that MS patients are more likely to be born in the spring months[6]. This has led some researchers to the hypothesis that longer durations of in utero vitamin D insufficiency, as is common during winter months, is related to the later development of MS. Reinforcing the notion of gestational susceptibility of MS, higher concordance rate are observed in dizygotic twins, who share the same intrauterine environment, compared to their nontwin siblings[7, 8]. A

small number of studies have investigated the potential relationship between in utero exposure to vitamin D and risk of MS; however, no study has examined the consistency of this data.

The purpose of this meta-analysis was to investigate the association between gestational vitamin D and risk of multiple sclerosis. The hypothesis was that vitamin D deficiency in utero is associated with an increased risk of multiple sclerosis in offspring.

## Methods

### Data Sources

A systematic literature search of the Embase and Pubmed databases was performed from inception to May 30, 2018 for identification of relevant articles. The Medical Subject Headings "Multiple Sclerosis", "Multiple Sclerosis, Relapsing-Remitting", or "Multiple Sclerosis, Chronic Progressive" were used in combination with “Mothers”, “Pregnancy”, “Infant, Newborn”, “Vitamin D”, “Fish Oils”, and free text terms “MS (Multiple Sclerosis)”, “maternal” or “gestational”, “vitamin D”, “serum”, “25-hydroxyvitamin D”, or “25(OH)D” were utilized in PubMed searches. The Emtree explosion terms ‘multiple sclerosis’, ‘mother’, ‘pregnancy’, ‘infant’, ‘vitamin D’, ’25 hydroxyvitamin D’, or ‘fish oil’, or the free text terms ‘maternal’, ‘gestational’, ‘25(OH)D’. Government reports, conference proceedings, and bibliographies of included articles were also searched.

### Study Selection

A study was selected for inclusion if it was an original, observational study that utilized an independent study population and provided relevant quantitative information. Included studies needed to investigate both clinically defined multiple sclerosis (in offspring) and vitamin D levels estimated or measured either in utero or shortly after birth. Studies were excluded due to lack of relevance, lack of

adequate information or inability to obtain quantitative information from study authors, duplicate study population as an included study, investigated season/month of birth without estimation of maternal vitamin D status during gestation, or if they were studies completed in animal models. Duplicate studies were also excluded.

**Data Extraction**

Studies fulfilling the inclusion criteria were independently reviewed and data was abstracted independently by two of the authors (E.A.J. and N.N.). All discrepancies were resolved by consensus. The following information was independently abstracted from each article: study authorship, year of publication, journal, study period, country, study design, inclusion and exclusion criteria, sample size, statistical analyses, and variables matched on or adjusted for. The following vitamin D information was collected: assessment in the mother or infant, time point of exposure assessment (e.g. first trimester, etc.), method of exposure determination (e.g. serum, dietary assessment, etc.), forms of vitamin D studied, and classification of vitamin D (e.g. quintiles, continuous, etc.). The method of ascertainment of the outcome, method of MS diagnosis, and type of MS were also collected.

**Assessment of Study Quality**

Newcastle-Ottawa Quality Assessment Scale (NOS) was employed to assess the quality of studies meeting the eligibility criteria[9]. The maximum points a study can receive is 9, based on selection of study population and study design.

**Statistical Analysis**

As most of the studies reported only adjusted estimates of risk in their populations, in which effect estimates were produced using models statistically adjusting for potential confounders, only

adjusted effect measures were analyzed. These adjusted estimates were pooled and random effects models with generic inverse variance methods were used to estimate a summary measure of effect in the main analysis and in stratified and sub-analyses[10]. Studies were weighted using the inverse variance method. Revman (Review Manager from the Cochrane Collaboration), a meta-analytic software, was utilized[11]. Clinical heterogeneity was assessed and reported. Statistical heterogeneity was assessed using the Woolf test for heterogeneity, where I-squared ($I^2$) values were calculated[12]. Funnel plots were used to assess the possibility of publication bias in all comparisons. For all measures, odds ratios (ORs), with 95% confidence intervals (CIs) were reported.

As there was significant heterogeneity in classification of vitamin D, the studies were analyzed based on vitamin D definitions. Since all 4 studies defined vitamin D in quintiles, the main analysis compared the highest quintile of vitamin D to the lowest quintile of vitamin D. Additionally, sub-analyses were completed using studies that defined the vitamin D exposure as a continuous variable. Two stratified analyses, based on whether vitamin D was measured in the mother or her offspring, were performed, one using the studies' quintile result and another using the results' from their continuous vitamin D analyses.



**Data Availability Statement**

The data supporting this meta-analysis are able from previously reported studies and datasets, which have been cited. The processed data are available from the corresponding author, EAJ, upon request.

## Results

**Description of the Studies**

The results of the literature search, the study selection process, and the number of studies included in the meta-analysis are reported in Figure 1. The systematic literature search resulted in 267

potentially relevant, published articles, 166 found via Embase and 101 identified using PubMed. Forty duplicate texts were excluded. Of the 227 studies assessed for eligibility, 94 were determined to be ineligible based on inclusion and exclusion criteria. One-hundred and thirty three articles were then assessed for further eligibility through screening based on title, with 40 being excluded as they did not investigate multiple sclerosis. Of the 93 remaining studies, 59 were excluded based on apparent lack of relevance during screening of the abstract.

The full text of the remaining 34 articles were reviewed in detail, with 4 studies meeting the inclusion criteria[8, 13-15]. The main study characteristics are shown in Table 1. All 4 studies were of case-control design and published prior to May 2018. One of the studies took place in Sweden, with the other three studies using populations within the United States, Denmark, and Finland. A total of 38,969 participants were included in this analysis, 1,346 of which had MS. Two of the studies assessed vitamin D levels in the mothers throughout their pregnancy[8, 13], while 2 studies measured vitamin levels in newborns shortly after birth[14, 15].

Most of the included studies determined vitamin D status through blood or serum samples; however one study estimated vitamin D using dietary assessment and a prediction model, developed in an independent population, that utilized race, dietary and supplemental vitamin D, body mass index (BMI), season of blood draw, laboratory batch, physical activity, alcohol intake, and hormone use to estimate serum vitamin D levels of mothers[8]. There was significant variability in the studies classification of vitamin D. While all studies classified vitamin D in quintiles, the cutpoints of these quintiles varied drastically between studies. Three studies also defined vitamin D in tertiles, commonly referred to as deficient, insufficient, sufficient; however, the specific classifications of these groupings also varied due to the lack of standardized, accepted definitions of acceptable levels of vitamin D in pregnant women. Finally, all 4 studies also defined vitamin D as a continuous variable. MS was commonly defined using the McDonald criteria and ascertained via registries or medical chart review. The average length of

follow-up time was approximately 30 years old, though this varied drastically between studies. Three studies performed matching while all studies adjusted for important covariates during analysis (Table 1).

**Quality of Included Studies**

Assessment of the quality of studies can be found in Table 2. Briefly, three studies had high quality while one study was of moderate quality. Potential bias stemmed from representativeness of cases, selection or definition of controls, and lack of reporting of non-response rates.

**Association of Vitamin D and Multiple Sclerosis**

When comparing the largest quintile of vitamin D to the smallest quintile of vitamin D level, higher levels of vitamin D were shown to have a protective effect against MS in random effects (OR: 0.63, 95% CI: 0.47, 0.84) models. Minimal heterogeneity was present in this analysis ($I^2$ = 30%). The funnel plot produced from this analysis was relatively symmetric (Figure 2). Due to concerns of bias, both via selection of study groups and in regards to vitamin D storage and measurement methods, as well as degradation over the study period, and the lower study quality of the Ueda et al study, sub-analyses were performed where the study was excluded. After exclusion of the Ueda et al study, a decreased risk of MS with an increasing amount of vitamin D was also found (OR: 0.56, 95% CI: 0.43, 0.74). Heterogeneity decreased upon exclusion of the Ueda study, as well ($I^2$ = 6%).

In sub-analyses completed using different definitions of the vitamin D exposure, results varied. The pooled estimates of effect when vitamin D was assessed as a continuous variable was not significant in the random effects model (OR: 0.77, 95% CI: 0.59, 1.01) (Table 3). However, there was significant heterogeneity in the analysis ($I^2$ = 85%). When the Ueda et al study was excluded, a significant odds ratio was found in random effects (OR: 0.69, 95% CI: 0.60, 0.79) analyses based on quintiles of vitamin D

(Table 3). Additionally, excluding the Ueda et al. study completely removed the heterogeneity in the previous analysis ($I^2$ = 0%).

For the analysis stratifying by individuals within which vitamin D was assessed, the quintiles of vitamin D were used, with the comparison being between the lowest and the highest quintiles in each study. Two studies assessed vitamin D in the mother[8, 13]; while two studies assessed vitamin D in the infant[14, 15]. The random effects model results show significant decreased risk of MS in offspring when vitamin D is assessed in the mother (OR: 0.57, 95% CI: 0.33, 0.97); however, this association is not significant when vitamin D levels were assessed in neonates (OR: 0.70, 95% CI: 0.46, 1.05) (Table 3). The test for subgroup differences revealed no differences between the two groups ($I^2$ = 0%), though publication bias may be possible. When using continuous vitamin D for an analysis stratified by which individual vitamin D was assessed in, the trend was similar: a significant decreased risk (OR: 0.68, 95% CI: 0.55, 0.83) of MS in offspring was found when vitamin D was assessed in the mother but the association was not significant (OR: 0.85, 95% CI: 0.60, 1.20) when it was assessed in the neonate.

## Discussion

In this first systematic literature review and meta-analysis of gestational vitamin D and risk of MS in the offspring, we identified lower levels of vitamin D during pregnancy are associated with an increased risk of MS for the offspring later in life. Specifically, when comparing the smallest quintile of vitamin D levels to the largest quintile using a random effects model, we found that the odds of developing MS was reduced by 37% for individuals who were exposed to the highest amount of vitamin D in utero compared to those who had been exposed to smallest amounts of vitamin D (OR: 0.63, 95% CI: 0.47, 0.84). The study results were robust to a variety of conditions including stratifying by study quality, study population, and by definition of the vitamin D exposure.

While the cause of the disease remains unknown, genetic and environmental factors, as well as their interactions, are likely contributors. In 1960, Sir Donald Acheson and his colleagues were the first to hypothesize the causal link between sunlight exposure and MS due to the unique latitude geographic distribution of MS prevalence[16]. This hypothesis was further refined to focus on the biologic mechanisms by which sunlight could influence MS risk, shifting the focus to vitamin D. From this theory, a substantial amount of evidence grew to support the argument for a causal relationship between vitamin D and MS risk. Previous studies have examined the relevance of UVB radiation, latitude vitamin D intake, and serum 25(OH)D levels in adolescence or adulthood[17-29]. In the studies that investigated vitamin D, an increased risk of MS in individuals with low levels of vitamin D and decreased risk of MS in those with high levels of vitamin D has been consistently found. Furthermore, a recent meta-analysis demonstrated that MS patients had lower mean levels of 25-hydroxyvitamin D compared to healthy controls[30]. Few studies have examined the possible association between vitamin D levels in utero and risk of MS later in life. Prior to this meta-analysis, results from these few studies were conflicting. Overall, our findings further demonstrate the protective effect of high vitamin D levels on the risk of MS in offspring. These results are supported by recent Mendelian randomization studies and a recent mouse study that further demonstrated the causal relationship between in utero vitamin D and MS[31-33].

This review and analysis has many strengths. Importantly, all included studies were of moderate-to-high quality, with three studies classified as high quality. MS requires numerous tests and specialist involvement prior to official diagnosis, thus misclassification in terms of MS status is highly unlikely. Additionally, the majority of studies in the analysis utilized biologic samples to estimate individuals' vitamin D levels. This ascertainment methodology increased the quality of the studies by reducing the possibility of misclassification and recall bias. Our study is strengthened by the fact that all included studies accounted for sex of the child and age or birth (month) date of the child, factors which are likely to confound the relationship between gestational vitamin D and MS. By controlling for these

factors, all included studies achieved high scores for comparability on the Newcastle-Ottawa Quality Assessment Scale NOS. Several studies also addressed numerous other variables including the latitude or region of birth and race/ethnicity. Finally, the use of similar methods of ascertaining cases and controls imparted higher quality on almost all of the studies. The meta-analysis was also strengthened by the inclusion of studies that utilized diverse populations from varying geographic regions.

However, due to the limitations and diversity of the primary studies, there are several limitations noted. First, three studies had to be excluded from the analysis due to a failure to provide adequate data[34-36]. While the funnel plots are relatively symmetrical, publication bias cannot be ruled out due to the small number of studies included. Furthermore, almost all included studies were population-based which lessens the likelihood that selection bias is driving the deduced association.

The major limitations of this analysis stems from the differences in how included studies collected and defined vitamin D levels. Since there is not a gold standard for classification of vitamin D, all studies had unique vitamin D definitions. Several studies measured vitamin D in nanomoles per liter, while other studies measured it in nanograms per milliliter. Furthermore, all studies analyzed this exposure differently, some defining it as a continuous variable, while others used categorical definitions. Even when studies classified vitamin D in the same manner, the measurement cutpoints varied between studies. Several of studies did not provide clear definitions of their exposure variable, further complicating the analysis. The variability in classifications and definitions of vitamin D could be an alternative, though unlikely, explanation for the findings of this meta-analysis.

Another important issue limiting this analysis is how well neonatal vitamin D levels represent vitamin D status during pregnancy, since two studies assessed vitamin D in infants. Infant vitamin D levels obtained through blood spots have been shown to be highly correlated with levels in umbilical cord blood[37] and moderately correlated with maternal vitamin D status at midgestation[38]. However, concentrations of vitamin D in neonatal blood are likely to be affected by the timing (hours after birth)

of serum collection and whether infants were fed prior to collection[39-41]. Correlation between newborn blood and umbilical cord blood or maternal gestational samples, could, therefore, vary based on these factors. Unfortunately, the two studies that assessed vitamin D status in the infants did not clearly state the points at which biologic samples were taken from the child. As such, it is impossible to know if these studies collected a measure of vitamin D that reflects the gestational period of these children. Finally, almost all included studies matched cases to controls or adjusted for numerous variables in the analyses, though these variables differed between studies. Nevertheless, adjusted effect estimates were used in this analysis because unadjusted data was rarely provided. This variation may have influenced the findings of each study and their comparability. Along with the differing definitions of vitamin D, this is a likely cause of the heterogeneity between studies.

## Conclusions

We observed an inverse association between gestational vitamin D levels and risk of MS, with higher levels of vitamin D during gestation or in neonates being associated with lower odds of offspring MS later in life. However, these results should be interpreted cautiously given the limitations of this meta-analysis and the included studies. Nevertheless, the high global prevalence of hypovitaminosis D among pregnant women[38, 42] and the fact that high maternal vitamin D levels could reduce both mothers' and their offspring's risk of MS provides additional rational for increasing vitamin D levels in pregnant women and for the continued research of a potentially modifiable risk factor (vitamin D) on risk of MS. In the future, standardized definitions or categories of vitamin D are necessary in order assure comparability of the studies and allow for pooling of study results. The variables used to match cases and controls, as well as the covariates adjusted for in analysis, will also need to be well thought out and similar across studies.

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Table 1: Characteristics of Studies Meeting Search Inclusion Criteria

| First author, year | Country | Study design | Total Sample Size (MS #) | Exposure assessed in | Vitamin D determined by | Vitamin D classification | MS definition | Outcome Ascertainment | Length of follow-up time | Matching | Confounders Adjusted |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mirzaei et al. 2011** | United States | Prospective nested case-control | 35,794 (199) | Mother-All trimesters | Prediction model composed of race, estimated dietary and supplemental vitamin D, BMI[A], season of blood draw, laboratory batch, physical activity, alcohol intake, hormone use | Quintiles, sextiles, and continuous variable | Unclear, physician-confirmed | Self-reported questionnaire, medical chart review, and physician-confirmed | 0 to 26 years | NA | Race/ethnicity, region[B], date of sample collection, diet, breastfed, smoking, BMI at age 18, age of child, mother's education, father's profession, mother's exercise, child's baseline vitamin d intake |
| **Munger et al. 2016** | Finland | Prospective nested case-control | 502 (176) | Mother-All trimesters | Serum (blood) samples | Quintiles, tertiles, and continuous variable | ICD-8 and 10 codes | Medical chart review | 18 to 27 years | On region[B], date of sample collection, mother's birth date, child's birth date | Date of sample collection, sex of child, gestational age |
| **Nielsen et al. 2017** | Denmark | Case-control | 1,451 (512) | Infant | Dried blood spots | Quintiles, tertiles, and continuous variable | McDonald and Poser criteria | Registry | 0 to 30 years | On child's birth date, sex of the child | Race/ethnicity, gestational age, birthweight |
| **Ueda et al. 2014** | Sweden | Case-control | 1,222 (459) | Infant | Dried blood spots | Quintiles and continuous variable | McDonald criteria | Registry and neurologist | 16 to 70 years | On region[B], sex of child, age of child by 5-year age groups | Region[B], child's birth date, sex of child, breastfed, sun exposure, vitamin D intake from dairy products, fatty fish consumption, smoking, BMI at age 20, Scandinavian parent, SES[C], MS family history |

[A] BMI: body mass index
[B] Region: includes latitude and/or residential area
[C] SES: socioeconomic status.

Table 2: Study Quality as Measured by the Newcastle-Ottawa Scale

| Study | Quality Score Part 1-Selection | | | | Quality Score Part 2-Comparability | Quality Score Part 3-Exposure/Outcome | | | Total Quality Score |
|---|---|---|---|---|---|---|---|---|---|
| | Adequate case definition | Represent-ativeness of cases | Selection of controls | Definition of controls | Study controls for sex and/or an additional factor | Ascertainment of exposure | Same ascertainment method for cases & controls | Non-response rate | |
| **Mirzaei et al 2011** | 1 | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 7 |
| **Munger et al. 2016** | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 8 |
| **Nielsen et al. 2017** | 1 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 7 |
| **Ueda et al. 2014** | 1 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 6 |

Table 3: Summary Odds Ratios for Studies of the Relation between Gestational Vitamin D and Risk of MS in Offspring

| | | Random Effect Model Pooled OR (95% CI) | $I^2$ values (*p* value) | Number of Studies |
|---|---|---|---|---|
| **Analysis using highest vs lowest vitamin D quintile** | | 0.63 (0.47, 0.84) | 30% (0.23) | 4[A] |
| **Analysis using highest vs lowest vitamin D quintile, excluding Ueda** | | 0.56 (0.43, 0.74) | 6% (0.35) | 3[B] |
| **Analysis using continuous vitamin D** | | 0.77 (0.59, 1.01) | 85% (0.0002) | 4[A] |
| **Analysis using continuous vitamin D, excluding Ueda** | | 0.69 (0.60, 0.79) | 0% (0.80) | 3[B] |
| **Analysis stratified by person vitamin D assessed in** (Highest vs lowest vitamin D quintile) | **Mother** | 0.57 (0.33, 0.97) | 51% (0.15) | 2 in mother[C] |
| | **Neonate** | 0.70 (0.46, 1.05) | 30% (0.23) | 2 in neonates[D] |
| **Analysis stratified by person vitamin D assessed in** (continuous vitamin D) | **Mother** | 0.68 (0.55, 0.83) | 0% (0.52) | 2 in mother[C] |
| | **Neonate** | 0.85 (0.60, 1.20) | 91% (0.0002) | 2 in neonates[D] |

[A] Mirzaei et al. 2011, Munger et al. 2016, Nielsen et al. 2017, Ueda et al. 2014
[B] Mirzaei et al. 2011, Munger et al. 2016, Nielsen et al. 2017
[C] Mirzaei et al. 2011, Munger et al. 2016
[D] Nielsen et al. 2017, Ueda et al. 2014

**Figure 1: Flow Diagram of Systematic Literature Search for the Meta-analysis**

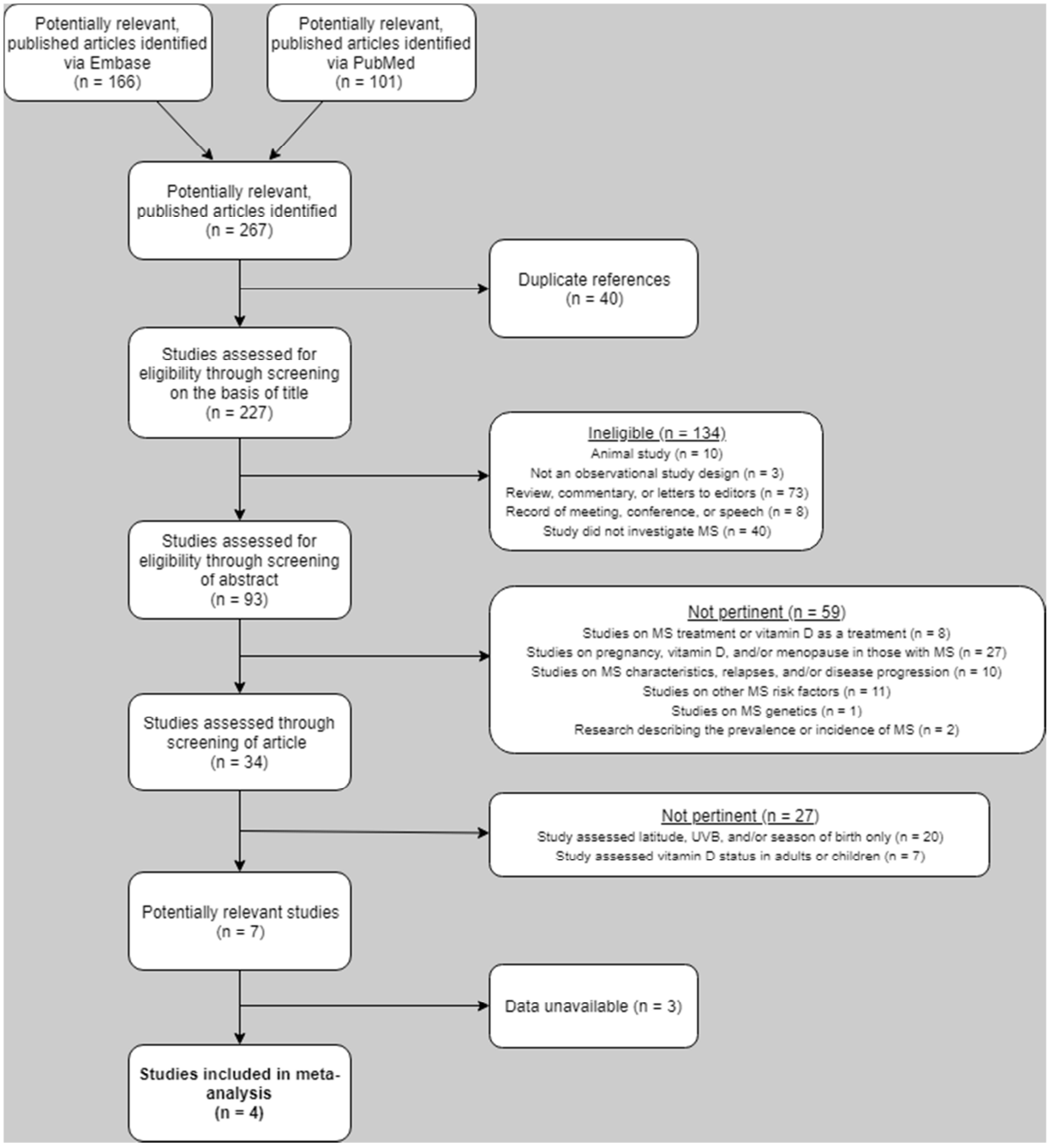

Figure 2: Funnel Plot of Gestational Vitamin D and Risk of Multiple Sclerosis (Highest Quintile vs Smallest Quintile of Vitamin D)