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Vitamin D Deficiency and Infertility: A Systematic Review

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Vitamin D Deficiency and Infertility:

A Systematic Review

Susanne Stoddard Sollis

An evidence based scholarly paper submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

James Kohl, Chair

College of Nursing
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ABSTRACT

Vitamin D Deficiency and Infertility: A Systematic Review

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Vitamin D regulates calcium and phosphorus homeostasis in the body. In recent years, studies have been conducted examining the role vitamin D plays in reproduction. Vitamin D deficiency is associated with infertility, decreased pregnancy rates, and hormonal changes. Asians appear to have a reverse correlation with pregnancy rates and vitamin D levels. There are some conflicting studies regarding vitamin D levels in relationship to infertility. This paper reviews the most recent literature focusing on the relationship between vitamin D status and infertility. This review evidences vitamin D levels >30 ng/mL result in improved fertility outcomes.

Keywords: vitamin D, deficiency, insufficiency, infertility, fertility, reproduction, in vitro fertilization, IVF, assisted reproductive technology, PCOS

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Vitamin D Deficiency and Infertility: A Systematic Review

Vitamin D is a fat-soluble secosteroid hormone involved in many functions of the body including calcium and phosphorous homeostasis, bone mineralization, cellular growth, and decreasing the risk for chronic illnesses such as diabetes, cardiovascular disease, cancer, obesity, and autoimmune diseases¹⁻⁶. Vitamin D receptors (VDR) facilitate the biological activity of vitamin D and are found in many cells throughout the body. VDR have been identified in reproductive tissues such as human testis, sperm, epididymis, seminal vesicle, prostate, ovaries, uterus, placenta, cervix, breast tissue, the pituitary, and hypothalamus^{1-3,5-12}.

Vitamin D has recently received attention for the role it plays in reproduction and fertility^{1-11,13-15}. Vitamin D deficiency has become a modern-day epidemic, being the most common nutritional deficiency worldwide^{6,16}. In the United States at least 1/3 of the population is vitamin D deficient (<20ng/mL), with 41.7% of adults between the ages of 20-64 being vitamin D deficient^{18,19}. Infertility is also quite prevalent affecting 15.5% of couples in the United States trying to conceive (95% CI: 8.6-27.5) and up to 53 million people worldwide^{20,21}. After a diagnostic workup for infertility, 15-30% of couples will be diagnosed with unexplained infertility²². One possibility for the unexplained infertility is vitamin D deficiency. Statistics suggest that among infertile women there is a high incidence of infertility. Li and colleagues¹⁴ found that 90.8% of women being worked up for infertility had insufficient (68.6% <32ng/mL) or deficient (22.2% <20ng/mL) vitamin D levels. Likewise, Ozkan and colleagues⁵ found in a population of infertile women 63% had vitamin D levels that were insufficient (36% 20-30ng/mL) or deficient (27% <20ng/mL). In addition, Anifandis and colleagues¹³ reported 79% of women undergoing in vitro fertilization (IVF) were vitamin D insufficient (48% 20.1-30ng/mL) or deficient (31% <20ng/mL). In men vitamin D deficiency has been associated with

decreased sperm motility ($P<0.05$). Consequently, vitamin D supplementation in men resulted in improved sperm function⁴.

The purpose of this systematic review is to evaluate the current research on vitamin D status and its relationship to infertility. Based on the findings, clinical recommendations will be made.

Methods

An electronic search was conducted to identify studies from 2010-2015 in the following databases: MEDLINE, CINAHL, and Academic Search Premier. Search terms included vitamin D, deficiency, fertility, and infertility. Articles were included if they were in English and dealt specifically with vitamin D and its relationship to fertility. Some articles prior to 2010 were included that had foundational research relating to vitamin D and infertility. A total of 15 studies met criteria for the review including prospective and retrospective cohort studies, prospective observational studies, prospective evaluations, retrospective studies, and cross-sectional studies. The 15 studies reviewed are summarized in Table 1 and focus on the most recent studies relating to vitamin D and infertility research done in humans.

Background

Vitamin D Role

Vitamin D is best known for its role in bone mineralization in the body via calcium and phosphorus homeostasis¹⁻⁵. Yet it is also involved in many other body functions including reproduction. Vitamin D is photochemically synthesized in the body from 7-dehydrocholesterol into cholecalciferol, or vitamin D₃. The other source of vitamin D in the body is ergocalciferol,

Table 1. Vitamin D Deficiency and Infertility						
Author/Title/ Location	Design	Purpose	Sample/ Population	Vitamin D Ranges/ Measurements*	Outcomes/Findings	Limitations
Aleyasin et al, 2011, Iran ¹ Predictive value of the level of vitamin D in follicular fluid on the outcome of assisted reproductive technology	Prospective cohort study	To assess the correlation between the levels of VD in FF and serum, and to determine whether the level of 25(OH)D is associated with ART outcomes.	82 infertile women (mean age 29.8 years) undergoing ART	Serum and FF 25(OH)D via enzyme immunoassay VD deficient <20 ng/ml VD insufficient 20-30 ng/ml VD sufficient >30 ng/ml	A significant correlation was found between 25(OH)D levels in serum and FF ($R= 0.767$, $P=0.001$). Fertilization rates decreased significantly ($P=0.018$) and implantation rates increased ($P=0.791$) with increasing levels of 25(OH)D. They concluded that VD deficiency does not play a pivotal role in the outcome of ART.	Study during low UV seasonal exposure, prevalent VD deficiency, grouped into tertiles rather than by VD status, small sample size, Asian population
Anifandis et al, 2010, Greece ¹³ Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome	Prospective observational study	To measure serum and FF 25(OH)D and glucose levels in women who underwent IVF/ embryo transfer and to see if those levels correlated with IVF success.	101 women who underwent IVF- intracytoplasmic sperm injection ovarian stimulation cycles	Serum and FF 25(OH)D via electrochemiluminescence immunoassay (ECLIA) VD deficient <20 ng/ml VD sufficient 20-30 ng/ml VD high >30 ng/ml	FF 25(OH)D had a significant negative correlation with embryo quality ($R=-0.27$, $P=0.027$). FF glucose levels were lower with high VD ($p=0.003$). Clinical pregnancy rates were lower with high VD ($P=0.047$). They concluded that high VD and low FF glucose have a negative effect on embryo quality and therefore IVF outcomes.	Small sample size, only 21% had VD levels >30 ng/ml
Blomberg Jensen et al, 2010, Denmark ³ Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract	Comprehensive analysis	To increase knowledge of the potential role of VD in male reproduction.	13 men who had orchiectomies due to testicular cancer or/and prostatectomies. Testis $n=13$, epididymis $n=7$, prostate $n=5$, seminal vesicle $n=3$, and semen samples $n=13$	N/A	There was marked expression of VDR and the VD metabolizing enzymes in human testis, ejaculatory tract and mature spermatozoa, which suggests that VD is important for spermatogenesis and maturation of human spermatozoa.	Small sample size, pathological specimens

<p>Blomberg Jensen et al, 2011, Denmark⁴</p> <p>Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa</p>	<p>Cross-sectional association study and in vitro study</p>	<p>To investigate the role of activated VD in human spermatozoa and whether VD serum levels are associated with semen quality.</p>	<p>300 young men (for semen quality and vitamin D serum levels)</p> <p>40 men (for the spermatozoa in vitro studies)</p>	<p>Serum 25(OH)D via isotope dilution liquid chromatography tandem mass spectrometry</p> <p>VD deficient <10ng/ml VD insufficient 10-19 ng/ml VD normal 20-30 ng/ml VD high >30 ng/ml</p>	<p>VD levels correlated positively with sperm motility and progressive motility ($P<0.05$). Men with VD deficiency had a lower proportion of motile ($P=0.027$), progressive motile ($P=0.035$), and morphologically normal spermatozoa ($P=0.004$) compared to men with high VD levels. Activated VD increased intracellular calcium concentration, sperm motility, and induced the acrosome reaction in mature spermatozoa suggesting VD contributes to optimal sperm function.</p>	<p>The role of VD in spermatozoa from infertile men needs further study</p>
<p>Corbett et al, 2004, New Hampshire, U.S.A.⁷</p> <p>Vitamin D: Is there a role in male infertility?</p>	<p>Prospective evaluation with qualitative and semi-qualitative analysis</p>	<p>To investigate the presence of VDR in human sperm.</p>	<p>Semen specimens from 11 fertile and 20 infertile men</p>	<p>N/A</p>	<p>VDR is present on the mid-piece in human sperm. VDR expression was inversely related to sperm concentration in infertile men versus fertile controls. There was a downward trend in VDR expression for patients with low motility, irrespective of fertility status.</p>	<p>Small sample size, mechanism of action of VD through the VDR on sperm has yet to be determined</p>
<p>Corbett et al, 2006, New Hampshire, U.S.A.⁸</p> <p>Vitamin D receptor found in human sperm</p>	<p>Prospective study with qualitative analysis</p>	<p>To investigate the presence of VDR in human sperm.</p>	<p>Semen specimens from 10 fertile men (mean age 33.7 ± 2.2 years)</p>	<p>N/A</p>	<p>VDR is present in sperm with its expression concentrated mainly in the head/nucleus in the post acrosome region and mid-piece of the sperm. VDR was present in all fertile subjects. Infertile men excluded from the study due to low or undetectable VDR expression, oligospermia, low motility, and poor morphology.</p>	<p>Small sample size, specimens from infertile men not included, role of VDR in human male fertility has not yet been determined</p>
<p>Firouzabadi et al, 2014, Iran²</p> <p>Value of follicular fluid vitamin D in predicting the pregnancy rate in an IVF program</p>	<p>Prospective observational study</p>	<p>To investigate whether there is a correlation between levels of 25(OH)D in the FF and the serum of infertile women and the results of IVF and rates of pregnancy.</p>	<p>221 infertile women (age 20-39 years)</p>	<p>Serum and FF 25(OH)D via enzyme immunoassay</p> <p>VD deficient <10 ng/ml VD insufficient 10-29 ng/ml VD sufficient ≥ 30-100 ng/ml</p>	<p>No significant correlation was seen between the pregnancy rate and the serum VD level ($P=0.094$) or the FF VD level ($P=0.170$). The serum and FF VD levels showed a significant correlation ($P=0.000$). In the group with sufficient VD levels there was a trend toward increased implantation and fertilization.</p>	<p>Asian population, prevalent VD insufficiency</p>

Garbedian et al, 2013, Canada ⁹ Effect of vitamin D status on clinical pregnancy rates following in vitro fertilization	Prospective evaluation	To investigate whether VD levels are predictive of implantation and clinical pregnancy rates in infertile women following IVF.	173 women (age 18-41 years)	Serum 25(OH)D VD deficient <20 ng/ml VD insufficient 20-30 ng/ml VD sufficient >30 ng/ml	Women with sufficient VD levels had significantly higher rates of clinical pregnancy per IVF cycle (52.5%) than the women with insufficient VD levels (34.7%) ($P<0.001$). Implantation rates were also higher in the sufficient group (34.5%) vs. the insufficient group (25.6%), but not statistically significant ($P=0.6$). Serum 25(OH)D levels may be a predictor of clinical pregnancy (OR 1.01, 95% CI 1.00-1.03)	Races classified as white, black, and other
Li et al, 2012, California, U.S.A. ¹⁴ Prevalence and risk factors of vitamin D deficiency in women with infertility	Retrospective review	To evaluate the prevalence of VD deficiency in patients presenting for infertility treatment and to identify risk factors that predispose low VD levels.	1192 women of reproductive age	Serum 25(OH)D VD deficient < 20 ng/ml VD insufficient: 20-32 ng/ml VD sufficient >32 ng/ml	The majority of women presenting with infertility had insufficient serum vitamin D levels (median=27ng/ml) with 68.6% being VD insufficient (<32ng/ml) and 22.2% VD deficient (<20ng/ml). High BMI, Asians, and those of black ethnicity are all risk factors for VD deficiency, with Asian women being at the highest risk.	Low UV seasonal exposure
Ozkan et al, 2010, New York, U.S.A. ⁵ Replete vitamin D stores predict reproductive success following in vitro	Prospective cohort study	To determine whether 25(OH)D levels in the FF of infertile women undergoing IVF demonstrate a relationship with IVF cycle parameters and outcomes	84 women of reproductive age	Serum and FF 25(OH)D VD deficient <20na/ml VD insufficient 20-30 ng/ml VD sufficient >30ng/ml	Serum and FF VD levels were highly correlated ($R=0.94$). Women with higher VD levels are significantly more likely to achieve clinical pregnancy following IVF ($P=0.013$). Appropriate supplementation of those deemed depleted of vitamin D might translate to improved fertility outcomes.	Small sample size, grouped into tertiles rather than by VD status
Paffoni et al, 2014, Italy ¹⁰ Vitamin D deficiency and infertility: Insights from in vitro fertilization cycles	Prospective cross-sectional study	To investigate IVF outcome in women with deficient 25(OH)D serum levels (<20 ng/ml) who had adequate ovarian reserve	154 women with serum 25(OH)D <20 ng/ml and 181 women with serum 25(OH)D \geq 20 ng/ml (age 18-42 years)	Serum 25(OH)D via chemiluminescence technology (DiaSorin) VD deficient <20 ng/ml VD insufficient 21-29 ng/ml VD sufficient \geq 30 ng/ml	Clinical pregnancy rates were 20% in the group with VD<20 ng/ml and 31% in the group with VD \geq 20 ng/ml ($P=0.02$). The adjusted odds ratio for clinical pregnancy when VD \geq 20 ng/ml was 2.15 (95% CI: 1.23-3.77). The group of women with the highest serum levels (>30 ng/ml) had the highest chance of pregnancy.	Primarily Caucasian

Rainer et al, 2012, Oklahoma, U.S.A. ¹⁵ Vitamin D deficiency and time to pregnancy in women with polycystic ovary syndrome	Retrospective review	To evaluate whether VD deficiency is associated with time to pregnancy in PCOS women	53 PCOS women who underwent ovulation induction with timed intercourse and/or intrauterine insemination. (age \leq 38 years)	Serum 25(OH)D VD deficient < 30 ng/ml VD sufficient \geq 30 ng/ml	There was a high prevalence of VD deficiency among PCOS women. No significant association was found between VD levels and time to pregnancy [HR=1.4 (95% CI: 0.3-8.5)].	Small sample size, all non-Hispanic, white females
Ramlau-Hansen et al, 2011, Denmark ¹¹ Are serum vitamin D levels associated with semen quality?	Cross-sectional study	To examine the association between low serum vitamin D concentration and estimates of male reproductive function.	347 young men from a pregnancy cohort designed to study the association between prenatal smoke and adult semen quality (age 18-21 years)	Serum 25(OH)D via isotope dilution liquid chromatography–tandem mass spectrometry VD deficient < 10 ng/ml VD insufficient 10-20 ng/ml VD sufficient >20 ng/ml	A high VD level was unexpectedly associated with lower crude median total sperm count and percentage of normal morphology sperm and a high level of crude median sex hormone-binding globulin and FSH. Adjusted free androgen index was lower at higher VD levels, and men with high VD had 11% lower free androgen index compared with men with low VD. These results do not indicate that low VD is a risk factor for poor semen quality.	Men were grouped into tertiles rather than by VD status, the “low vitamin D” group included anyone with VD < 25 ng/ml, not enough VD deficient men (only 6%) to detect an effect
Rudick et al, 2012, California, U.S.A. ⁶ Characterizing the influence of vitamin D levels on IVF outcomes	Retrospective cohort study	To validate other investigators' findings of higher IVF pregnancy rates in women sufficient in VD and to clarify the role of VD in reproduction among a diverse group of women.	188 infertile women undergoing IVF	Serum 25(OH)D via radio-immunoassay (DiaSorin) VD deficient < 20 ng/ml VD insufficient: 20-30 ng/ml VD sufficient >30ng/ml	VD deficiency is associated with lower pregnancy rates in non-Hispanic whites ($P=0.04$), but not in Asians. Asians had lower pregnancy rates when VD sufficient ($P=0.01$). The VD deficient were younger ($P=0.01$), heavier ($P=0.03$), and less likely to have diminished ovarian reserve ($P=0.01$). VD status did not correlate with ovarian stimulation parameters, fertilization rates, or embryo quality.	Small sample size of Asians, so the reverse correlation could be a chance finding

<p>Rudick et al, 2014, California, U.S.A.¹⁶</p> <p>Influence of vitamin D levels on in vitro fertilization outcomes in donor-recipient cycles</p>	<p>Retrospective cohort study</p>	<p>To explain the role of VD in reproduction by looking at the relationship between recipient VD levels and pregnancy rates in donor-recipient IVF cycles.</p>	<p>99 female recipients of egg donation undergoing IVF and embryo transfer</p>	<p>Serum 25(OH)D via radio-immunoassay (DiaSorin)</p> <p>VD deficient < 20 ng/ml VD insufficient: 20-30 ng/ml VD sufficient >30ng/ml</p>	<p>Adjusted clinical pregnancy rates were lower among VD deficient recipients than among VD replete recipients (37% vs 78%). Live-birth rates were 31% among VD deficient recipients compared with 59% among VD sufficient recipients. Lower VD levels were associated with lower pregnancy rates in recipients of egg donation. This study suggests that VD may be mediated through the endometrium.</p>	<p>Primarily Caucasian</p>
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VD: vitamin D, 25(OH)D: 25-hydroxyvitamin D, ART: assistive reproductive technology, FF: follicular fluid, IVF: in vitro fertilization, VDR: vitamin D receptor

*For ease in comparison all units were converted to ng/ml and rounded to the nearest whole number.

or vitamin D₂, which comes from the diet. In the body 80-90% of vitamin D is a result of skin exposure to the UVB rays from the sun, while only 10-20% is acquired from the diet^{12,23-27}. It is estimated that between 5-30 minutes of sun exposure, without sunscreen, just twice a week between 10:00 AM and 3:00 PM may be adequate in maintaining sufficient vitamin D levels. Sun exposure time varies depending on skin color/sensitivity, amount of skin exposed (arms and legs recommended), season, time of day, altitude, and latitude^{18, 24}. Sunscreen use of SPF 15 can reduce vitamin D₃ synthesis by 99%²⁴. Dietary forms of vitamin D include fortified foods (milk, orange juice, yogurt), fatty fish (sardine, salmon, tuna), calf liver, egg yolks, yeast/fungi (mushrooms), and supplements^{24,25,27-29}.

Vitamin D is inactive when it is first synthesized in the body via sun exposure or diet in the form of D₂ or D₃. The inactive form of vitamin D is then metabolized by the liver to 25-hydroxyvitamin D, or 25(OH)D, which is the level most often used to assess vitamin D status. 25-hydroxyvitamin D is metabolized by the kidneys into its active form, 1,25-dihydroxyvitamin D, also known as calcitriol^{12,23-25,27-30}. The calcitriol then binds to and activates the VDR in the target cells, which have been found in many reproductive organs and tissues throughout the body^{1-3,5-12,30}. In addition to VDR expression, vitamin D activating and inactivating enzymes are necessary in regulating the cellular response to vitamin D^{3,29,30}. Calcitriol is tightly regulated through negative feedback controls determined by parathyroid hormone, calcium, and phosphorus levels^{24,29,31}.

Vitamin D Status Definitions

One limitation of the research involved with vitamin D is the ability to make adequate comparisons due to variability in the authors' definitions of vitamin D status. See Table 2.

References	Deficient	Insufficient	Sufficient
Aleyasin ¹	<20 ng/mL	20-30 ng/mL	>30 ng/mL
Anifandis ¹³	<20 ng/mL	-----	20-30 ng/mL
Blomberg Jensen ⁴	<10 ng/mL	10-19 ng/mL	20-30 ng/mL
Firouzabadi ²	<10 ng/mL	10-29 ng/mL	≥ 30-100 ng/mL
Garbedian ⁹	<20 ng/mL	20-30 ng/mL	>30 ng/mL
Li ¹⁴	<20 ng/mL	20-32 ng/mL	>32 ng/mL
Ozkan ⁵	<20 ng/mL	20-30 ng/mL	>30 ng/mL
Paffoni ¹⁰	≤20 ng/mL	21-29 ng/mL	≥30 ng/mL
Rainer ¹⁵	<30 ng/mL	-----	≥30 ng/mL
Ramlau-Hansen ¹¹	<10 ng/mL	10-20 ng/mL	>20 ng/mL
Rudick ⁶	<20 ng/mL	20-30 ng/mL	>30 ng/mL
Rudick ¹⁶	<20 ng/mL	20-30 ng/mL	>30 ng/mL

Although there are variations in the definitions, for the most part the ranges are similar.

Table 3 lists the most common vitamin D reference ranges and the definitions used in this paper.

Vitamin D Level	Vitamin D Status
<20 ng/mL	Deficient
20-30 ng/mL	Insufficient
>30 ng/mL	Sufficient

Variability was also present in the unit of measurements for vitamin D levels. Vitamin D levels are usually reported in ng/mL or nmol/L. For ease in comparison in this review all vitamin D levels were converted to ng/mL and rounded to the nearest whole number. Vitamin D levels can be measured in follicular fluid or a serum sample. Several studies compared vitamin D levels between the two measurement methods and found a significant correlation ($P=0.001$)¹ ($P=0.000$)² ($P<0.001$)⁵ ($P<0.001$)¹³, indicating that either method can be used with similar results.

Results

Animal Studies – Females

Much of the early research done linking vitamin D to its role in reproduction was done in animal studies. In studying vitamin D deficiency in female rats, Halloran and Deluca³² discovered that vitamin D deficient females were capable of reproducing, although their overall fertility was reduced by 75% compared to vitamin D sufficient females. This was due to a reduced likelihood of impregnation and an increased likelihood of pregnancy complications³². Multiple studies have confirmed smaller litter sizes (by 30-40%) and impaired neonatal growth in the offspring of vitamin D deficient mothers³²⁻³⁴.

There is some evidence that vitamin D deficiency and its effects on fertility may be an indirect effect. Vitamin D deficiency usually leads to hypocalcemia and hypophosphatemia. Without vitamin D, the body absorbs up to 30% less calcium and 20% less phosphorus²⁴. When the calcium and phosphate deficiencies in animals were supplemented with a diet high in calcium, phosphate, and lactose (to improve calcium absorption), the vitamin D levels still remained low. Yet, when the hypocalcemia and hypophosphatemia were corrected in the female mice and rat studies, their fertility returned. These results suggest that vitamin D deficiency may affect infertility indirectly due to the disruption of vitamin D function in maintaining calcium/phosphorus homeostasis. It's possible the primary cause of infertility may be hypocalcemia and/or hypophosphatemia³⁴⁻³⁶.

Yet there is also evidence that a reduction in vitamin D levels contributes directly to infertility due to physiological changes. For example, in vitamin D receptor (VDR) female null mutant mice (mice that have been genetically modified to not have the vitamin D receptor)

gonadal insufficiencies such as uterine hypoplasia and impaired folliculogenesis were observed. Additionally, there was a 24% decrease of aromatase activity and expression in the uterus compared to wild-type mice. Aromatase is a key enzyme in regulating estrogen biosynthesis in the ovary and uterus. Calcium supplementation increased the aromatase activity (only to 60% of that in wild-type animals, $P=0.005$) and restored fertility in some. Yet luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels remained abnormal, indicating that calcium supplementation only partially corrects the problem³⁷.

Animal Studies – Males

Animal research in male subjects further demonstrated the role of vitamin D in reproduction. Male rats fed a vitamin D deficient diet were compared to those fed a vitamin D replete diet and were then mated with age-matched, vitamin D sufficient females. Although the males fed a vitamin D deficient diet were still able to reproduce, they were much less effective. Vitamin D deficient rats had a 45-55% reduction in successful mating, which was defined as the presence of sperm in the vaginal tract^{38,39}. There was also a 73% reduction in fertility, or successful pregnancies, in vitamin D sufficient females inseminated by vitamin D deficient males compared to the females inseminated by the vitamin D sufficient males³⁸. Male rats fed a vitamin D deficient diet also displayed a reduction in testicular and epididymal sperm count, Sertoli cell dysfunction, a reduction in Leydig cell number, and degenerative changes in germinal epithelium⁴⁰. Fertility could be restored in vitamin D deficient rats by supplementation with vitamin D (cholecalciferol), calcitriol, or calcium³⁹.

Furthermore, physiological changes have been observed in VDR null mutant male mice, which can contribute to infertility. For example, these mice exhibited gonadal insufficiencies such as decreased sperm count and motility, histological abnormalities of the testis, and a

reduction in aromatase and gene expression in the testis and epididymis (58% and 35%, respectively). Some VDR null mutant mice had fertility restored after calcium supplementation, but their levels of LH and FSH remained elevated, indicating an abnormal endocrinological state³⁷.

Human Female Reproduction

Vitamin D insufficiency or deficiency is present in 58% to 91% of women with infertility^{6,14,16}. Some risk factors for vitamin D deficiency in women include an elevated body mass index (BMI), polycystic ovarian syndrome (PCOS), Asian ethnicity, and those of black ethnicity^{9,14,15}. In comparison to women who were vitamin D sufficient, BMI was significantly higher in women with vitamin D insufficiency ($P=0.02$)⁹. As vitamin D is a fat soluble vitamin, it is thought that with more adipose tissue vitamin D is stored and not as easily accessible for the body to use^{9,21}.

VDR expression is present in the pituitary, hypothalamus, ovary, uterus, and placenta, suggesting a definite physiologic role for vitamin D in female reproduction^{3,6,10,12}. Vitamin D is also involved in the regulation of several hormones in the body including the anti-Müllerian hormone (AMH), follicle stimulating hormone, estradiol (a type of estrogen), and progesterone, all having to do with fertility^{2,12}. AMH is a good indicator of ovarian reserve¹². In the endometrium, calcitriol (the active form of vitamin D in the body) binds to the VDR to regulate target genes. HOXA10 is one of the target genes fundamental in uterine receptivity to embryo implantation^{5,6,10,16,25}. Another target gene regulated by calcitriol is CYP19, which encodes aromatase, an essential enzyme in the production of estradiol^{2,6}. Estrogen and progesterone both play a significant role in reproduction including regulation of the menstrual cycle,

folliculogenesis, ovulation, luteinization, implantation, and endometrial decidualization to maintain the pregnancy^{5,12}.

Human Male Reproduction

In men, vitamin D receptors (VDR) and vitamin D metabolizing enzymes have been found in mature spermatozoa, testis, epididymis, prostate, seminal vesicles, Leydig cells and the ejaculatory tract^{3,7,8,11}. These findings led Jensen and colleagues³ to propose that vitamin D plays an important role in spermatogenesis and maturation of human spermatozoa. VDR expression in sperm is located primarily on the head/nucleus and mid-piece, possibly indicating a role for vitamin D in mitochondrial DNA function and/or head nuclear function. VDR expression was diminished in men with low sperm motility^{7,8}.

In addition, vitamin D appears to be integral for optimal sperm function. A positive correlation ($P < 0.05$) was found between serum vitamin D levels and the percentage of total motile sperm and progressive motile sperm. In comparison to vitamin D sufficient men ($VD > 30$ ng/mL), men with vitamin D deficiency ($VD < 10$ ng/mL) had lower proportions of motile ($P = 0.027$), progressive motile ($P = 0.035$), and morphologically normal ($P = 0.004$) spermatozoa. Likewise, a positive correlation ($P < 0.03$) was noted between serum calcium levels, which vitamin D regulates, and the percentage of total motile sperm ($P = 0.028$) and progressive motile sperm ($P = 0.027$). Administration of calcitriol was found to increase intracellular calcium concentrations (by 5-10 fold), sperm motility ($P = 0.010$), and induced the acrosome reaction ($P = 0.024$) in mature spermatozoa⁴.

In Vitro Fertilization (IVF) Studies

An IVF population provides an environment in which the effects of vitamin D can be studied in many aspects of reproduction. Five recent studies showed statistically significant higher rates of pregnancy following IVF when vitamin D levels were sufficient ($P=0.013$)⁵ ($P=0.04$)⁶ ($P<0.001$)⁹ ($P=0.03$)¹⁰ ($P=0.004$)¹⁶. This was true for all ethnic groups except for Asians, whose vitamin D status demonstrated a negative correlation with pregnancy rates ($P=0.01$)⁶. Two studies showed no significant correlation between pregnancy rates following IVF and vitamin D levels ($P=0.959$ and $P=0.094$). Of note, these studies were both done in primarily Asian populations with prevalent vitamin D deficiency^{1,2}. One study done in Greece (geographically close in proximity to Asia) demonstrated decreased pregnancy rates as vitamin D levels increased in combination with lower glucose levels ($P=0.047$). They concluded that increased vitamin D levels have an effect on insulin, altering glucose metabolism, ultimately resulting in poor IVF outcomes¹³.

Embryo quality in relationship to vitamin D was examined in several studies. Paffoni and colleagues¹⁰ found that women with sufficient vitamin D were more likely to obtain top quality embryos ($P=0.006$) with increased frequency of embryo transfer at the blastocyst stage. Garbedian and colleagues⁹ demonstrated that women with sufficient vitamin D were more likely to undergo embryo transfer on day 5 (71.8%) vs. day 3 (58.9%) ($P=0.054$). Day 5 transfers are more ideal and dependent on the number of good quality embryos, indicating that women with higher vitamin D levels may have higher quality embryos⁹. These studies contrast the findings of Anifandis and colleagues¹³ who found mean embryo score quality, but not the cumulative embryo score, to be lower in women who were vitamin D sufficient ($P=0.009$) suggesting that higher vitamin D levels in combination with lower glucose levels decreases the possibility of

achieving pregnancy. This was a smaller study with only 21% of the subjects being vitamin D sufficient¹³, so these results may not be representative of a larger population.

Limitations in the Research

There are several limitations of the research on vitamin D deficiency and infertility. Many of the studies were conducted in animals and it is unclear if the same levels of vitamin D in rats and mice correlate with the levels in humans. There are also a limited number of human studies, smaller sample sizes in the studies that have been done, and variability in vitamin D status definitions and measurements.

More human studies with larger sample sizes will give us further insight into the relationship of vitamin D and infertility. Additional studies focusing on vitamin D supplementation and fertility outcomes when sufficient vitamin D levels are maintained in both men and women attempting to conceive would be beneficial. Further studies are also needed in an infertile human population to establish ideal calcium dosing/levels to improve fertility.

Conclusion

The findings from this systematic literature review suggest that vitamin D status has a significant impact on fertility and reproduction. These findings are important because, although there are many factors that can cause infertility, vitamin D deficiency may be an explanation for some of the unexplained infertility cases or a contributor to the other causes of infertility. Also because vitamin D influences calcium and phosphorus levels these should be monitored in conjunction with vitamin D.

Clinical Implications

Vitamin D deficiency can have a negative effect on the fertility of both men and women in all races studied, with the exception of Asian populations^{1-11,13-15}. With these findings we recommend that vitamin D status be included as part of a standard infertility workup and considered in those of reproductive age planning to get pregnant. Vitamin D supplementation and/or sensible sun exposure is our recommendation in men and women who are deficient or insufficient in all races except for Asians. Vitamin D is a relatively safe and inexpensive supplement. Although there was a wide variety of definitions for vitamin D status, it seems that a serum 25(OH)D >30 ng/mL demonstrated the best outcomes^{2,4-6,9,10,16}. Calcium supplementation revealed fertility benefits in several animal studies^{34-37,39} and should also be considered; 1000 mg daily is the recommended intake⁴¹.

Supplementation of at least 600-800 IU of vitamin D₂ or D₃ daily should be used for maintenance in those with sufficient vitamin D levels (>30 ng/mL), although doses of 1500-2000 IU daily may be necessary to maintain sufficient levels⁴¹⁻⁴³. Patients with insufficient vitamin D levels (20-30 ng/mL) should be dosed according to their level. On average 100 IU of vitamin D daily will increase serum 25(OH)D levels by 0.7-1.0 ng/mL⁴¹. It is important to keep in mind that patients who are obese, have malabsorption syndromes, or have medications that affect vitamin D metabolism may require two to three times the amount of vitamin D to maintain adequate levels⁴². In adults with deficient vitamin D levels (<20 ng/mL) we recommend supplementing with 50,000 IU of vitamin D once weekly for 8 weeks, or until serum vitamin D levels are >30 ng/mL. Once vitamin D levels are sufficient, they should be maintained with a dose of 1500-2000 IU of vitamin D daily^{41,42}. Serum 25(OH)D levels should be checked after 3

months of therapy to ensure sufficiency⁴¹. Sufficient vitamin D levels could possibly increase a couple's fertility and likelihood of pregnancy, along with other health benefits.

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