

The Role of Vitamin D in Fertility and Gestational Development:  
Eastern and Western Perspectives

A Capstone Project

Submitted in partial fulfillment of the requirements for the degree  
Doctor of Acupuncture and Oriental Medicine

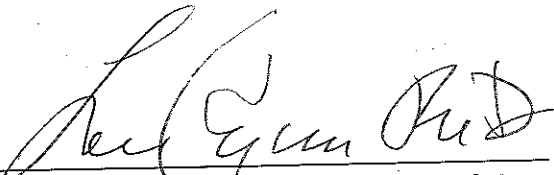
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## Abstract

A great deal of research and theorization has been generated regarding factors influencing human fertility and gestational development. Complementary medicine, including the acupuncture and herbal treatment methods of Traditional Chinese Medicine, is often sought out and concurrently used with allopathic medicine by individuals seeking reproductive assistance. The impact of serum vitamin D levels on physiological functioning is one factor that has been and continues to be investigated for its impact on the reproductive system as well as for overall health. The current study utilized grounded theory method to review existing literature for the purpose of generating a clearer understanding of vitamin D's influence in fertility and gestational development from both Western and Eastern medical perspectives. Qualitative data were compiled and analyzed to discover common themes as well as differences in medical and philosophical perspectives.

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## Chapter One: Introduction

Vitamin D is in vogue. A significant proportion of the world's population is affected by hypovitaminosis D, that is, with insufficient serum levels for optimum health (Holick, 2011; Lips, 2007; Mithal, Wahl, Bonjour, Burckhardt, Dawson-Hughes, Eisman, El-Hajj Fuleihan, Josse, Lips, & Morales-Torres, 2009). The issue is expected by some to be a widespread health issue for the next century (Grayson, and Hewison, 2011). A plethora of scientific scrutiny has attempted to unravel the complex influences this substance exerts on human morbidity. Recent scientific thought has reclassified vitamin D as a prohormone (with several active metabolites which behave as hormones) elucidating a broader understanding of its function and influence within human physiological processes. (Berkow, 1992). Yet despite recent findings and widespread evidence of population deficiencies, the recommended daily allowances (RDA) for dietary and supplemental intake have remained unchanged for decades (Zitterman, 2003). One must question then, the impact of a known insufficient serum level on quality of life.

Suspected links exist between insufficient serum vitamin D levels and the development (or the lack of prevention) of several disease processes. (Grant, & Boucher, 2011; Zaidi, 2010; Zitterman, 2003). Sufficient levels may thereby be supposed to optimize various aspects of homeostasis. Optimal physiological functioning is desired to facilitate reproduction of the species and vitamin D levels may therefore affect several factors that influence fertility and live birth rates. Replete levels are challenging to maintain in modern times due a wide variety of factors: latitude, age, ethnicity, cultural practices, dietary shifts and low vitamin D



food content, urbanization and pollution, global weather pattern shifts, sunscreen use, chronic disease states, and gender (Mithal, et al., 2009; Lapp, 2009; Zitterman, 2003; Rajakumar, Greenspan, Thomas, & Holick, 2007; Bosomworth, 2011). The discovery of vitamin D receptors (VDR) in a wide variety of human tissues as well as the various health associations of their particular polymorphisms with human pathology is fostering deeper understanding of vitamin D's complex effects on optimal homeostatic functioning, disease states and the challenges these receptor variants may present in maintaining replete levels (Paneierakis, Gouielmos, Mamoulakis, Maraki, Papavasiliou, & Galanakis, 2009; Roff, & Wilson, 2008, Clendenen, Arslan, Koenig, Enquist, Wirgin, Agren, Lukanova, Sjodin, Zeleniuch-Jacquotte, Shore, Hallmans, Toniolo, & Lundin, 2008; Amato, Pacini, Aterini, Punzi, Gulisano, & Ruggerio, 2008).

Traditional societies may have advocated practices that ultimately resulted in repletion levels, even if the active substance we now know as vitamin D was not identified. Indigenous medical systems such as Traditional Chinese Medicine (TCM) may have essentially treated any insufficient levels by identifying symptom patterns associated with insufficient vitamin D levels by virtue of their understanding of the human body and its functioning in relationship to the environment, rather than the biochemical processes occurring per se. In eastern medical thought, the relationship between a person's body, mind, spirit, lifestyle and the environment is crucial to observe in order to rectify any emerging pathological imbalances. Identification and corrections of imbalances within this relationship would theoretically also optimize reproductive capacity and functioning. Eastern

medicine consequently historically has provided very different diagnostic and treatment protocols than western medicine approaches for the same or similar pathological issues. This investigation hopes to further elucidate the connection in TCM practices to western ones by addressing vitamin D level effects in the area of reproduction and gestational development.

In the language and philosophy of TCM, developed from detailed observations of interactions between human beings and nature, how can light metabolism diagnostically viewed? China, the birthplace of TCM, is located between 18°15'N and 53°30' N latitude—a very large range ([www.worldatlas.com](http://www.worldatlas.com)). Considerable variation in sunlight patterns occur in a country of this size. Solar ray strength has been shown to be affected by several factors including the path length the rays must pass through the ozone layer of the atmosphere, the amount of pollution or ozone in the atmosphere, the location of the sun's zenith in relation to a particular geographic location (influencing the angle of intensity of the solar rays reaching the earth), latitude, season, and time of day (Holick, 1995). In the winter months, the zenith angle of the sun increases and the amount of solar radiation reaching the earth diminishes to varying degrees based on the above factors. Less solar radiation in the UV B range (290-315nm) is thus available in the fall and winter months for the photolysis of 7-dehydrocholesterol to precholecalciferol in human skin. Precholecalciferol is a relatively unstable compound which converts to cholecalciferol (vitamin D<sub>3</sub>), and is then acted on in the liver and kidney to yield calcitrol and 24,25(OH)<sub>2</sub>D, the active forms of vitamin D. (Zitterman, 2003; Holick, 2007). By virtue of its latitude, the potential for a negative health impact exists in

China that may have been addressed in TCM treatment protocols and diagnostic disease pattern discrimination.

The history of vitamin D in the western world is well documented at least as far back as the 19<sup>th</sup> century. In the early 19<sup>th</sup> century and into the early part of the 20<sup>th</sup> century, children afflicted with rickets were commonplace in Europe and North America. Rickets is caused by severe vitamin D deficiency resulting in bone softening, weakness, and bending of the long bones in the legs under the body's weight. Western medical practice found oral vitamin D administration and exposure to UV (ultraviolet) light to be curative of the condition. The modern daily intake levels of vitamin D supplementation are based on the amounts needed for prevention of rickets or osteomalacia and assume adequate skin exposure to sunlight occurs (Holick, 1995). In general, dietary supplementation becomes necessary for one of two reasons—to maximize cellular function if the dietary intake is low, or to achieve a desired physiological effect by correcting cellular function as a result of an ongoing disease process (Zeisel, 2000). The recommended dietary allowance (RDA) of vitamin D was calculated as all RDA's are, from a bell shaped curve of the needs of the population in its totality. For vitamin D, the current RDA is 400 IU per day. This strategy does not allow for differences in lifestyle, dietary habits, geography, skin pigmentation or race, normal aging, gender, genetics, or pathology present within individuals (Zeisel, 2000). Nor does it consider optimum serum circulating levels of activated vitamin D. Hepatic hydroxylation of vitamin D precursor ingested or activated via sunlight produce 25-hydroxyvitamin D (25[OH]D) as a major metabolite that can be easily measured in the serum. Levels

highly correlate with the amounts of circulating active vitamin D, making this a good marker to look at to assess vitamin D status in an individual. Serum ranges have no consensus on what constitutes an adequate level for disease prevention, but the following are often used for guidance:

**Table 1: Serum 25(OH)D Levels and Corresponding Morbidities**

<u>Serum 25[OH]D levels</u>	<u>Associated issues</u>
Deficiency (less than 25nmol/L)	short latency diseases like rickets, osteomalacia
Insufficiency (25-75 nmol/L)	long latency diseases like osteoporosis, fractures
Optimal (75-110 nmol/L or more)	health

(Bosomworth, 2011)

Latitude alone has a major influence on the amount of activated vitamin D formed via this route. A study in Boston, Massachusetts, latitude 42° N, found solar photoactivation of the 7-dehydrocholesterol is possible from March to November, leaving at least three months of inadequate solar exposure yearly strictly due to geographical location of residence. In Bergen, Norway, latitude 60° N, the range narrows to April to October, and due to the change in the sun's zenith angle, a full 100% decrease in the peak amounts of solar activated previtamin D is produced (Webb, Kline, & Holick, 1988). The furthest north state in the United States is Alaska, spanning from latitude 71° N at its northernmost point-- where the sun sets for a full month in the winter, and whose return is highly celebrated in January—to Amatignak Island in the Aleutian chain at 51°16' N ([www.worldatlas.com](http://www.worldatlas.com)). This latitude range just barely overlaps with the northernmost latitude of China, and encompasses a vast range of geography nearly equivalent to that of China's latitude

range, merely further north. Individual populations living above latitude 35° are at risk of insufficiency in serum vitamin D levels due to lack of adequate UVB rays during the winter months—October/November to March (Holick, 2011; Zaidi, 2010). Vitamin D levels required via supplementation or diet would understandable be different in a location such as Barrow, Alaska, as compared to Los Angeles, California with individuals having a range of dietary requirements based on area of residence, lifestyle and physiological factors.

Today, vitamin D's impact on optimal human health is beginning to be understood to encompass much more than calcium metabolism—VDRs (vitamin D receptors) have been found in many tissues and cell types including intestinal, muscle, pancreas, immune system, liver, skin, pituitary, placenta and ovarian cells (Zitterman, 2003). The existence of multiple receptor sites implies the importance of this substance in optimum physiological functioning on several levels at once. Exactly what role those receptors play in physiological functioning is yet to be clearly understood in many instances. As can be seen from the above list of cell types, effects potentially affect numerous organ systems. Of particular interest for this study is VDR presence within the reproductive system. Any substance that affects more than one physiological system, and particularly the reproductive system, likely has had a major evolutionary impact. Most living beings, whether plant, animal or single celled organism, have the ability to utilize the wavelengths of sunlight to regulate calcium metabolism and the production of calcium based structure. They also all have the ability to reproduce. In the field of human reproduction, the impact of hormonal balance is known to influence fertility and

gestational development. Emerging research indicates the prohormone effects of vitamin D potentially play a role in reproduction at multiple levels. Research into the effects of Traditional Chinese Medical treatments has likewise begun to show positive influences for many issues causing infertility. As an acupuncturist, a student of reproductive medicine and a resident of a far northern climate, my curiosity regarding the overlap of these fields is the catalyst for the initial inquiry this project provides into this question. Clients in diverse age groups, including those of reproductive age, are nearly all deficient in vitamin D levels in this locale when serum is tested (personal observation). What role does the presence of VDRs in reproductive tissues indicate with regards human reproductive functioning and fertility? Did Eastern medical theory and practice encompass this possible effect by virtue of its holistic relational approach to human life interacting with nature?

### **Research Objective**

This research study was undertaken to discern the answer to the question “What is the role of vitamin D deficiency on human fertility and gestational development from both Western and Eastern medicine perspectives?”. The next chapter provides the literature review that establishes the foundation for this study.

## Glossary of Terms

### Abbreviations:

- 1,25-OH-D      1,25-dihydroxyvitamin D<sub>3</sub>
- 7-DHC          7-dehydrocholesterol
- 25-OH-D        25-hydroxy vitamin D
- AR              Androgen
- ASRM          American Society of Reproductive Medicine
- BMI             Body mass index
- BV              Bacterial vaginosis
- EPDS          Edinburgh Postnatal Depression Scale
- IVF             In vitro fertilization
- NK              Natural killer cells
- PBMC         Peripheral blood mononuclear cells
- RDA            Recommended daily allowance
- RPL            Recurrent pregnancy loss
- SNP            Single nucleotide polymorphism
- TCM            Traditional Chinese Medicine
- TNF-alpha     Tumor necrosis factor alpha
- UV             Ultraviolet
- VDD            Vitamin D deficiency
- VDR            Vitamin D receptor
- With/out       With or without

Definitions:

- Hormone—chemical substance produced in one area of the body that controls and regulates activity of certain cells or organs in another area of the body.
- Leptin—a hormone secreted by adipose tissue, positively associated with increased cancer risk.
- Polymorphisms—the presence of two or more distinct phenotypes in a population due to the expression of different alleles of a given gene; the existence of an organism in several forms or color variations.
- Prohormone—a physiologically inactive hormone precursor; an intraglandular precursor of a hormone.
- Replete—abundantly supplied; complete.
- Vitamin—fat or water soluble organic substances essential in minute amounts for normal growth and activity of the body; obtained naturally from plant and animal foods.

([www.dictionary.reference.com](http://www.dictionary.reference.com), [www.merriam-webster.com](http://www.merriam-webster.com) )



## Chapter Two: Review of Literature

### Overview

This chapter provides a summary of the literature pertinent to the objectives of this study's inquiry. Chapter two first lays the groundwork for following sections by providing a historical perspective of vitamin D, its physiology, and influential factors in its *in vivo* production such as latitude and diet. The bulk of the chapter is dedicated to the review of studies that pertain to both Western and Eastern medical perspectives of the role of vitamin D in reproduction. Both human and animal studies are included. The chapter culminates with a literature review integration section that sums up current knowledge and establishes the need and direction for the current study, laying the groundwork for future study possibilities.

### Historical Perspective

The existence of phytoplankton dates to more than 750 million years from present history. The coccolithophor *Emeliani huxleii* is an example of a modern species existing largely unchanged from its prehistoric counterparts. Like its ancestors and many other modern plants, this phytoplankton makes ergosterol, a substance that is converted to vitamin D<sub>2</sub> when exposed to sunlight. As evolution proceeded, the vertebrates emerged and were able to successfully live on dry land. Vitamin D played a major role in the ability of these animals to produce a calcified skeleton once outside of the calcium rich ocean environment and thus maintain structure and locomotion capabilities outside of the liquid environment (Holick, 1995). The ability to produce vitamin D (in animals vitamin D<sub>3</sub>) was retained in the skin (Holick, 2007). Vitamin D has been utilized by living organisms since the

beginning of the evolutionary process on planet earth to transform the energy of the sun into physical structure. Prehistoric humans initially had all of their skin exposed to sunlight; overtime more clothing was used, enabling habitation at more northern climates and due to shifting cultural and moral codes of conduct until approximately 5% of skin is routinely exposed today (Vieth, 1999). In modern human beings, the vital nature of vitamin D to health became glaringly apparent in the 19<sup>th</sup> thru 20<sup>th</sup> centuries in Europe and North America. This was the time period of the industrial revolution—housing construction of slum tenements very closely together blocked out sunlight in the streets below and accelerated coal burning blocked out daylight from the thick pollution layer in the air. Skin exposure to UV light in the B spectrum (290-315 nm) as well as oral vitamin D intake was shown to not only cure rickets, but prevent it as well (Holick, 1995; Zitterman, 2003). Vitamin D fortification of foods such as milk, cereals and was instituted as well as recommendations for sunlight exposure that effectively turned the tide of the rickets epidemic (Eliot, 1926; Palm, 1890; Rajakumar, 2003; Rajakumar, Greenspan, Thomas, & Holick, 2007). Fortification was originally established to correct for environmental deficiency (sunlight) rather than dietary intake deficiency (Vieth, 1999). This was the widely held view of the importance of vitamin D and its metabolites for many years—the regulation of calcium metabolism alone.

### **Physiological Processes**

The current recommended dietary allowance (RDA) for vitamin D dietary intake for adults in the United States is 200 IU per day. This value is based on recommendations from the National Research Council in 1989 (National Research

Council, 1989; Holick 1995). Many researchers consider this level controversial; of these most consider this amount to be far too low. The RDA of a substance is generally determined based on the needs of the entire population, rather than taking into account such individual factors as lifestyle, environment, normal aging differences, developmental, racial or genetic issues. A particular RDA is not necessarily reflective of the needs of a particular individual in a particular location at a particular time (Zeisel, 2000). In the case of vitamin D, latitude, cultural practices, and dietary habits play a tremendous role in the amount of circulating vitamin D in any one individual. Vitamin D deficiency is generally defined as a serum 25-hydroxyvitamin D level under 25nmol/L (10ng/mL); vitamin D insufficiency ranges from under 40-80nmol/L; and desirable circulating levels have been variable among sources but ranging from 50-100nmol/L (Martini & Wood, 2006). Supplementation raises the serum level only 1 ng/mL per 100 IU ingested (Holick, 2008).

In obese individuals, chronic deficiency levels are common due to the efficiency with which vitamin D is stored in fat, decreasing bioavailability (Sellar, 2005). Obese individuals can only raise their serum levels of vitamin D at approximately half the rate of their normal counterparts, whether from sunlight exposure or dietary supplementation. The increased adipose tissue associated with an increased BMI leads to increased leptin secretion, which attenuates the gene expression of two important hydroxylases in activating vitamin D forms (Maetani, Maskarinec, Franke, & Cooney, 2009). One proposed theory is a displacement of

25(OH)D by leptin, causing obese individuals to be less efficient at raising serum vitamin D levels (Maetani et al., 2009).

In the United States, overall levels of 25(OH)D were found to be lower in the 2000-2004 National Health and Nutrition Examination Survey (NHANES) compared to the levels found in the same survey for 1988-94. In the 1988-1994 survey, 18,158 people were tested by radioimmunoassay; in the 2000-2004 survey, 20,289 people were tested. Overall mean serum levels were lower in the 2000-2004 group for age-standardized 25(OH)D levels by 5-20nmol/L in people older than 12 years old. In one subgroup of adults, non-Hispanic whites, BMI, milk intake and sun protection appeared to have an influence consistent with the trend seen in serum levels (Looker, Pfeiffer, Lacher, Schleicher, Picciano, & Yetley, 2008).

### **Latitude and Vitamin D**

Latitude has a major influence on the amount of vitamin D activation in the skin as it influences the strength of the sun's rays during the various seasons. The further north in latitude a person lives, the less intense the amount of sunlight is received in the fall, winter and spring months with respect to the relatively constant sunlight exposure of those individuals residing at the equator. In the winter season, vitamin D activation is compromised in latitudes above 35° (Holick, 2011). The pathway of the sun's light is increasingly more tangential the further north one goes. This causes an increase in the absorption of the UV-B wavelength of light required for vitamin D activation as the light passes thru the ozone layer of the atmosphere (Rajakumar et al., 2007). An in depth study of the effects of latitude, season and time of day were conducted in the 1980's in Boston, Massachusetts (latitude 42° N),

and Edmonton, Canada (latitude 52° N), in comparison to Los Angeles, California (latitude 34° N), and San Juan, Puerto Rico (latitude 18° N). In June and July, the peak of summer months, cutaneous activation of 7-dehydrocholesterol to precholecalciferol was at its peak of production in Edmonton and Boston. By August, however, production showed a gradual decline until October. In Boston less than 4 % of 7-dehydrocholesterol production occurred between the hours of 1130 and 1430 Eastern Standard Time (EST) and by November, there was essentially no production detected at any time of day. This continued till March when a gradual increase in detection began till the summer months. Similarly, in Edmonton 7-dehydrocholesterol production decreased after the summer months. However, being further north than Boston, no detectable levels of activated vitamin D were found beginning mid October and continuing through April, after which a gradual increase began. The two locations these results were compared to, Los Angeles and San Juan, both showed precholecalciferol production year round (Webb et al, 1988). The half life for 25(OH)D in blood circulation is one to two months, so by the middle of winter the probability of insufficient circulating serum levels is high in northern latitudes (Vieth, 1999). Oral vitamin D supplementation remains in the blood stream for hours rather than days as sunlight activated vitamin D does. Bound to chylomicrons and lipoproteins in the blood stream, orally supplemented vitamin D is efficiently removed by the liver and stored in adipose tissue after a few hours (Vieth, 1999).

## **Diet and Vitamin D**

Dietary habits contribute to the deficient vitamin D levels found in many populations for a variety of reasons. Vitamin D is fat soluble and its absorption is maximized when taken as food or with it. Natural dietary sources for vitamin D are limited and not routinely eaten in many modern cultures. Foods include cod liver oil, oily fish such as salmon, sardines and mackerel, beef and cooked eggs. Fortified sources of vitamin D include milk, some orange juice, cereal, and margarine (Martinti et al., 2006). In modern times concerns about obesity and cardiovascular health with regard to cholesterol levels and dietary fat intake have caused many individuals to severely limit their intake of fatty foods. Cod liver oil is no longer routinely utilized as a supplement thus effectively decreasing a potentially large dietary vitamin D contribution from days gone by. Farmed salmon, the form most individuals have access to in their diet, contains between 10-25% the levels of vitamin D as its wild counterparts (Holick, 2008). Populations living in Arctic areas where sunlight exposure is minimal have been shown to be replete for vitamin D when they consume a traditional diet rather than processed foods. Traditional diets are highly based on animal foods. In the Arctic consumption of land and sea mammals (caribou, salmon, whale, birds, wild bird eggs, etc) is the majority of foodstuffs consumed on a daily basis (Kuhnlein & Receveur, 2007). As far back as the 1950's medical personnel in the Arctic observed no vitamin D deficiencies despite the long dark winters and lack of UV light exposure in populations that ate a largely native diet and whose children were breastfed (Schaefer, 1959).

### **Vitamin D and Reproduction—Western Perspective—General Considerations**

Nesby-O'Dell et al., examined vitamin D levels in African American and White women of reproductive age in the United States who participated in the third National Health and Nutrition Examination Survey (NHANES III). This survey was conducted in 1988-1994 (spring and fall) and the women examined provided an extensive interview, medical exam and blood samples. Hypovitaminosis D was defined as serum levels of  $\leq 37.5$  nmol/L of 25[OH]D; a lower level would have required a larger sample size to establish any significance in the results per the authors. Excluded from the sample to be analyzed were women whose data were incomplete: no serum 25[OH]D level was available, food diary information was missing, vitamin D supplements were taken, oral contraceptives used, smoking status or body mass index (BMI) was not reported. The final sample size was 1546 African American women and 1426 white women. Age, seasonality of data collected, rural versus urban residence, and income level data were also analyzed. Among African American women 42.4% were below 37.5 nmol/L; among white women 4.2 % were below this threshold. For both races, levels were lower in the spring months and higher in the fall. For African American women significant independent associations were found between examined variables and vitamin D hypovitaminosis results. The variables examined were milk and cereal consumption, vitamin D supplementation, season, residence location, BMI and current use of oral contraceptives. For white women significant associations were found with season, BMI, and consumption vitamin D supplements. The authors note that upwards of 50% of the African American women in the United States could

enter pregnancy in a state of vitamin D deficiency (Nesby-O'Dell, Scanlon, Cogswell, Gillespie, Hollis, Loker, Allen, Dougherty, Gunter, & Bowman, 2002).

Another study utilizing NHANES data from 2001-2006 looked at vitamin D levels in women 13-44 years old (i.e. of childbearing age) and found women in their first trimester had 25[OH]D levels similar to their nonpregnant counterparts despite the fact that more of them were taking vitamin supplementation (61% versus 32%). Third trimester pregnant women had higher levels than first trimester women, perhaps due to a longer time period for supplementation to occur. Most supplements utilized by participants in these study years delivered 400 IU per day of vitamin D. This study was unable to control for seasonality due to the methods of data collection utilized in this time period. Again, the largest association was made between race and 25[OH]D levels—non-Hispanic white women had much higher 25[OH]D levels than minorities in both pregnant and nonpregnant groups. Overall, 41% of women had 25[OH]D levels < 50nmol/L and 75% had levels < 75nmol/L. Since maternal levels affect both mother and child, and vitamin D can cross the placenta, optimal circulating levels for pregnant women are important to ascertain and maintain. While exactly what that level should be remains a debate, a general consensus of serum 25[OH]D levels of  $\geq 50$ nmol/L are necessary for children and adults to prevent skeletal abnormalities and up to 75 nmol/L for the prevention of non-skeletal pathologies (Ginde, Sullivan, Mansbach, & Camargo, 2010). Similarly, a study conducted in south eastern United states in pregnant women less than 14 week gestation found 97 % of Blacks, 81% of Hispanics and 67% of Caucasians in the study to be at deficient (<50 nmol/L) or insufficient (>50 and <80 nmol/L)



serum levels of 25(OH)D. Race was found to be the most likely association with insufficient or deficient vitamin D levels, as well as first pregnancy status (Johnson, Wagner, Hulsey, McNeil, Ebeling, & Hollis, 2010). Pigmented skin has been shown to require 3-6 times as long to achieve production equilibrium for skin activation of vitamin D compared to fairer skinned counterparts (Vieth, 1999).

Vitamin D levels have also been implicated to influence the reproductive capacity in men as well. A recent study by Pilz et al. found increased testosterone levels in men supplemented with vitamin D as compared with placebo (Pilz, Frisch, Koertke, Kuhn, Dreier, Obermayer-Pietsch, Wehr, & Zitterman, 2011).

### **Animal Studies**

Animal studies have been utilized to foster understanding of the mechanisms involved in vitamin D's impact on fertility. Kwiecinski, Petrie, and Deluca (1989) investigated the effect of vitamin D deficiency on male rats by feeding two groups of rats either a replete or a deficient diet once they were weaned to adulthood. Both groups were then mated to age matched, vitamin D replete females. The male group with the insufficient vitamin D diet was able to reproduce with the replete females. However, the likelihood of the birth of a live, normal litter was 27% of that when the females were mated with vitamin D replete male rats. Normal females mated with normal males having live healthy litters were around 76% of pregnancies, but normal females mated with deficient males had a 40% chance of a healthy litter birth following confirmed pregnancy. Pregnancy issues with the deficient male pregnancies included abortion, stillbirth, maternal demise in childbirth and fetal resorption or false pregnancy. Further, the likelihood of successful mating as

shown by sperm in the vaginal smears taken in the female rats during estrus was 45% that of the replete males. This study did not differentiate whether this effect was due to hypocalcemia or vitamin D levels and thus further more detailed scrutiny is required. The authors speculated that spermatogenesis fails in rats with vitamin D deficiency (Kwiecinski, Petrie, & Deluca, 1989).

Halloran and Deluca performed a complementary experiment (1980). Female weanling rats were fed either a vitamin D replete or deficient diet till maturity, and then mated with vitamin D replete males. Once again reproduction was possible—but litter sizes were 30% smaller, and overall fertility was 75% reduced in the deficient females compared to replete females. Litter gestational development appeared normal but growth was retarded (Halloran & Deluca, 1980).

Male and female vitamin D receptor negative mice were investigated for reproductive abnormalities (Kinuta, Tanaka, Moriwake, Aya, Kato, & Seino, 2000). Vitamin D receptor null (i.e. having an inability to have vitamin D to bind to any cell in the body) male mice were examined and found to have abnormal testes histology, decreased sperm counts, decreased sperm motility, and significantly lowered aromatase activity in the testes and epididymis, 58% and 35% respectively to VDR normal mice. The female mice displayed uterine hypoplasia and abnormal folliculogenesis. Both males and females had hypergonadotropic hypogonadism confirmed with elevated LH and FSH levels. Estradiol supplementation corrected the histological abnormalities in the gonads of both sexes. Restoring calcium levels to normal partially corrected the hypogonadism and elevated the aromatase activity to 60% of the VDR normal mice. The authors concluded vitamin D was necessary for

optimal gonadal functioning in both sexes of mice (Kinuta, et al., 2000). Data from other researchers showed stimulation of aromatase gene expression in immature sertoli and testicular germ cells in rats when vitamin D analogues are administered (Zanatta, Bouraima-Lelong, Delalande, Silva, and Carreau, 2011).

Vitamin D receptors have been found in numerous body tissues. In a rat study, VDR proteins were found to be most abundant in the granulose cells of follicles undergoing atresia, theca cells of the corpus luteum and the theca cells of the primary and secondary follicles. Moderate VDR expression was found on the surface endothelium and weak expression found in the ovarian stroma and blood vessels. While this study focused on the possible links between VDR and androgen receptors in ovarian cancer and possible inhibitors or stimulators of tumor growth in these abnormal conditions, the identification of a large number of VDR in the ovary tissues indicates an important role in the optimal functioning of this organ, but does not elucidate whether this is due to calcium homeostasis effects of perhaps epigenetic gene regulation (Ahonen, Zhuang, Aine, Ylikomi, & Tuohimaa, 2000). This confirmed the results found by Yoshizawa et al. in 1997 when female VDR null mice were found to have alopecia, uterine hypoplasia and impaired folliculogenesis, not present in merely vitamin d deficient mice (Yoshizawa, Handa, Uematsu, Takeda, Sekine, Yoshihara, Kawakami, Arioka, Sato, Uciyama, Masushige, Fukamizu, Matsumoto, & Kato, 1997). Gestational vitamin D deficiency was found to cause permanent brain changes in rats in a literature summary review (Levenson, & Figueroa, 2008). The human and rodent brain have been found to make 1-alpha-hydroxylase which activates 25(OH)D, and VDRs have been found in central

nervous systems of both species. Gestational vitamin D effects appeared causally linked to permanent changes in gene expression in the brain. Further research was indicated to determine the degree to which these influences affect adult behavior and neurological diseases (Levenson et al., 2008). The effects of developmental deficiency of vitamin on rat the rat brain showed offspring from deficient females had exhibited increased lateral ventricle volumes, reduced differentiation, decreased expression of neurotrophic factors, and mildly distorted brain shapes. The authors concluded vitamin D could act as a neurosteroid, directly affecting brain development and may be an explanatory risk factor for the development of neuropsychiatric disorders (Eyles, Feron, Cui, Kesby, Harms, Ko, McGrath, & Burne, 2009).

In a mouse knock out model for the enzyme responsible for activating vitamin D, 1 alpha-hydroxylase, the expected issues with calcium metabolism were expressed, namely hypocalcemia, secondary hyperparathyroidism, etc. Female mice also exhibited uterine hypoplasia, decreased ovarian size, compromised folliculogenesis, anovulation and infertility not corrected by normalizing calcium levels. Male mice had diminished fertility that was correctable by calcium level normalization (Panda, Miao, Tremblay, Sirios, Farookhi, Hendy, & Goltzman, 2001; Uhlund, Kwiecinski, & DeLuca, 1992).

Cardiac issues due to gestational vitamin D deficiencies in rats were investigated by a group of researchers in Australia. Adult female rats were randomly separated into two groups and fed a vitamin D replete or deficient diet for 6 weeks, then switched to a growth version of the same diets for two weeks before

breeding and pregnancy. On postnatal day 3, litters were reduced randomly to 8 offspring. The culled rats were decapitated and their hearts immersion fixed for later testing. The remaining offspring were killed by decapitation (for enzymatic cardiocyte isolation) or anesthetized and perfusion-fixed (for stereological cardiomyocyte number determination). Data analysis showed no impact of maternal vitamin D levels on body weight, heart size or cardiomyocyte number in the day 3 offspring (when cardiomyocytes are actively proliferating). By 4 weeks of age this proliferation has effectively ceased and the vitamin D gestationally deficient group of weanlings showed delayed cardiomyocyte maturation, left ventricle hypertrophy, and an increase in cardiomyocyte number and size indicating vitamin D's role in cellular differentiation likely altered normal development (Gezmish, Tare, Parkington, Morley, Porrello, Bubb, & Black, 2010).

A review of animal experimental studies, human descriptive studies, human observational analytic studies and human experimental studies by Lucas et al for Nutritional Reviews in 2008 summarized the role of insufficient vitamin D prenatally or perinatally in human or rat offspring. Studies included in the review showed possible impaired ovarian function and spermatogenesis, reduced induction of implantation immune tolerance, pre-eclampsia, shorter gestational intervals, lower birth rate, shorter knee-heel length, brain function alteration, associations with schizophrenia development, multiple sclerosis, type 1 diabetes, Crohn's Disease, and asthma (Lucas, Ponsonby, Pasco, & Morley, 2008). These possible effects were echoed in relationship to concern's about the vitamin D status of mothers during breastfeeding and the impact on the infant—impacting bone health,

cardiovascular health, immune function, and glucose metabolism (Will, Taylor, & Wagner, 2009).

### **Human Studies—Fertility Related**

Classically vitamin D functions in an endocrine fashion, regulating calcium homeostasis in the tissues via mechanisms in the kidney. Evans, Bulmer, Kilby, and Hewison in 2004 delved into some of the non-classical actions of the vitamin, involving the placenta-- an extra renal tissue capable of synthesizing active vitamin D. Levels of VDR and 1-alpha-hydroxylase are highest in first trimester decidua suggesting a role in the immune modulation required for successful implantation (Evans, Bulmer, Kilby, & Hewison, 2004). Ovarian cells in mice have been shown to contain VDR, as does the hypothalamus, and ovarian cells in vivo accumulate 1,25-(OH)<sub>2</sub>D<sub>3</sub> (Jones, Strunell, and Deluca, 1998). VDR numbers increase during the estrus phase and decrease during the proestrus phase, implying a role in ovarian function (Jones et al., 1998). Macrophages as well are capable of activating to the 1,25-(OH)D form (Holick, 2008).

Somigliana et al. (2007) looked at vitamin D status in relationship to the presence or absence of endometriosis. 140 consecutive female patients undergoing surgery at the Department of Obstetrics and gynecology in the “Fondzione Ospedale Maggiore Policinico Mangiagalli e Regina Elena” who were of reproductive age were selected and evaluated for endometriosis and 25-hydroxyvitamin D<sub>3</sub> levels. Since vitamin D is an immune system modulator and women with endometriosis often have immune system irregularities, the investigators hypothesized vitamin D might play a role in the implantation of endometrial cells in the peritoneal cavity.

Endometrial tissue has been found to be capable of extra-renal activation of 25-(OH)D to the biologically active 1,25-(OH)<sub>2</sub>D<sub>3</sub>. Women with endometriosis were found to have higher levels of 25-(OH)D, and the level found was associated with the severity of their endometriosis staging. The authors recommended further study to specify possible confounding factors, but postulate the increased 25-(OH)D levels may cause local immune modulation in the peritoneal cavity allowing endometrial implants to gain a foothold and proliferate (Somigliana, Panina-Bordignon, Murone, Di Lucia, Vercellini, & Vigano, 2007).

In the early 1990's, vitamin D metabolites in serum and follicular were examined in 10 healthy women undergoing in-vitro fertilization and embryo transfer in Israel. This was the first time vitamin D metabolites had been documented within the follicular fluid, and the levels found there were statistically significantly correlated with serum levels for 1,25-(OH)<sub>2</sub>D<sub>3</sub> (P<0.001), as well as 25-OHD<sub>3</sub>, and 24,25-(OH)<sub>2</sub>D<sub>3</sub> (P<0.01) and rising estradiol levels during gonadotropin induced ovarian stimulation (Potashnik, Lunenfeld, Levitas, Itskovita, Albutiano, Yankowitz, Sonin, Levy, Glezerman, & Shany, 1992).

Sperm have also a positive correlation between vitamin D levels and sperm analysis parameters. Vitamin D levels were positively correlated with sperm motility (P< 0.0005), and men with deficient levels of serum 25(OH)<sub>2</sub>D<sub>3</sub> had lower amounts of motile, progressively motile and morphologically normal sperm in comparison to replete level males (P=0.027, 0.035, and 0.44 respectively). The results were thought to be due to a role for vitamin D in calcium metabolism

intracellularly (Jensen, Bjerrum, Jessen, Nielsen, Joensen, Olesen, Petersen, Juul, Dissing & Jorgensen, 2011).

### **Human Studies—Gestational Development/Pregnancy Related**

In pregnancy serum levels of  $1,25(\text{OH})_2\text{D}$  rise in the first trimester and fall to normal levels soon after delivery in normal pregnancy. The levels measured do not change significantly for singleton versus twin pregnancies and the levels do not follow any other normal known fluctuations in pregnancy related hormones (Reddy, Norman, Willis, Goltzman, Guyda, Solomon, Philips, Bishop, & Mayer, 1983). In an in vitro experiment, decidual natural killer cells were treated with either  $1,25(\text{OH})_2\text{D}_3$  or its precursor ( $25(\text{OH})\text{D}_3$ ) for 28 hours. A decreased production of cytokines (including interleukin 6, tumor necrosis factor, etc) occurred along with an increased production of mRNA for an antimicrobial peptide cathelicidin (Evans, Nguyen, Chan, Innes, Bulmer, Kilby, & Hewison, 2006). Elevated placental proinflammatory cytokine release, especially of tumor necrosis factor alpha (TNF-alpha) is associated with miscarriage, preterm labor, and preeclampsia. Calcitriol inhibits the TNF-alpha mediated production of inflammatory cytokines through a process thought to be regulated by a vitamin D receptor in trophoblasts (Diaz, Noyola-Martinez, Barrera, Hernandez, Avila, Halhali, & Larrea, 2009).

Vitamin D deficiency and insufficiency has been found to be common in southern latitudes where repletion would be expected to occur more frequently (Johnson et al., 2010).  $25(\text{OH})\text{D}$  levels drawn at less than 14 weeks gestation in 154 African American, 194 Hispanic, and 146 Caucasian women showed ninety-seven percent of African American pregnant women, 81% of Hispanics and 67% of



Caucasians had levels of deficiency or insufficiency--<80nmol or <32 ng/mL. Race was the most important factor for vitamin D insufficiency or deficiency in this sample population. (Johnson, et al., 2010). The link between vitamin D levels and post partum depression was investigated by Murphy, Mueller, Hulsey, Ebeling, and Wagner (2010). An exploratory, descriptive analysis of each of 97 postpartum women was performed and the women were followed thru seven monthly visits. At each visit, the participants were drawn for serum 25(OH)D testing and concurrently administered a demographic questionnaire and the Edinburgh Postnatal Depression Scale (EPDS), a screening tool used to identify women with postpartum depression symptoms. Results were grouped according to vitamin D levels of insufficient ( $\leq$  32ng/mL) or sufficient ( $>$ 32ng/mL). The study concluded 75% of the women were insufficient for serum levels of 25(OH)D. When season and vitamin D supplementation were controlled for in the data, a significant relationship was found between high EPDS scores (i.e. more depressive symptoms) and low vitamin D levels for the first seven months postpartum ( $p=0.02$ ). The authors found these results need to be replicated with a larger sample size and controlling for other possible confounding factors as well as needing a controlled sample where women without postpartum depressive symptoms are included as well (Murphy, Mueller, Hulsey, Ebeling, & Wagner, 2010).

An untested hypothesis has been generated regarding a possible link between serum vitamin D levels with autism developmental risk. Autism rates are higher in genetic hypomelanomic skin disorders; the authors of the hypothesis therefore wish to test if this is due to behavioral influences (i.e. staying out of the sun) or epigenetic

or genetic variants producing hypomelanosis also increase in some way reproductive capacity and thus increase the proportions of affected genes in the gene pool (Bakare, Munir, & Kinney, 2011).

Grayson and Hewison (2011) reviewed the effects of vitamin D on mineral homeostasis regulation, implantation, infection, and inflammation during pregnancy. They found the following issues have been associated with insufficient vitamin D intake in pregnant women:

**Table 2: Maternal/Fetal Issues Associated with Vitamin D Insufficiency**

	Maternal Issues	Fetal/Neonatal Issues
Clinical Problem	Preeclampsia Bacterial Vaginosis Gestational Diabetes	Small for Gestational Age Fetal bone/skeletal issues Neonatal/childhood Bone Mass Asthma Type 1 Diabetes Multiple Sclerosis Autism Maternal-fetal HIV transfer

(Grayson & Hewison, 2011)

The authors concluded the need for further research in expanded clinical trials, and propose the link between vitamin D level and its influence on inflammation and

infection may be broadly involved in the maintenance of pregnancy (Grayson & Hewison, 2011).

Researchers at the USDA Western Human Nutrition Research Center and the University of California, Davis are currently recruiting participants to assess inflammatory markers and pregnancy outcomes in vitamin D supplemented women at risk of vitamin D deficiency. This study expects to conclude in 2013 (Stephensen, <http://clinicaltrials.gov>). Research into the effects of vitamin D level in autoimmune disease and antiphospholipid antibodies in pregnancy is also currently under way in Europe with an eye focused on whom to supplement and when (Meroni, Tincani, Alarcon-Riquelme, Schoenfeld, & Borghi, 2009).

Vitamin D deficiency (VDD) during pregnancy has been found to be associated with an increased incidence of bacterial vaginosis (BV) infections in the first trimester of pregnancy (Bodnar, Krohn, & Simhan, 2009). Bacterial vaginosis infections during pregnancy are associated with adverse outcomes and can lead to miscarriage or fetal morbidity. In this study, the incidence of BV in women less than 16 weeks gestation decreased as 25(OH)D levels increased—57% of women with levels of less than 20 nmol/L were positive for BV, compared with 23% having 25(OH)D levels of 80nmol/L or higher ( $P<0.001$ ) (Bodnar et al., 2009). In nonpregnant females, however, douching smoking, and black race were associated with BV infection; oral contraceptive use was inversely associated with BV (Hensel, Randis, Gelber, & Ratner, 2011).

The relative effects of vitamin D deficiency for pregnant women and neonates are varied according to a 2010 article discussion. Maternal effects in the case of

severe deficiency (<10ng/mL serum 25(OH)D) can include an increased risk of preeclampsia, impaired calcium absorption, bone loss, poor weight gain, myopathy, and elevated parathyroid hormone levels. This same level in the infant born of these women showed infants small for gestational age, hypocalcemia, seizures, heart failure, enamel defects, large fontanelle, congenital rickets, or rickets of infancy if breastfed. At the insufficient level (11-32ng/mL serum 25(OH)D) maternal effects included bone loss and subclinical myopathy; the effects in neonates born of these mothers were hypocalcemia, decreased bone mineral density, and rickets of infancy if breast fed. At levels of toxicity (>100ng/mL serum 25(OH)D) mothers showed increased urine calcium loss and hypercalcemia, the infants also showed hypercalcemia (Mulligan, Felton, Riek, & Bernal-Mizrachi, 2010). Maternal vitamin D insufficiency can affect fetal bone development as early as 19 weeks gestation (Mahon, Harvey, Crozier, Inskip, Robinson, Arden, Swaminathan, Cooper, & Godfrey, 2010).

Vitamin D binding to the VDR in a target tissue initiating gene transcription may be the mechanism of how all these effects are set in motion. Epigenetic alterations in the normal vitamin D catabolism in pregnancy may play a role in vitamin D availability at the fetal/placental interface, affecting implantation and placental development. In an in vitro cell culture experiment human placental, trophoblasts, and chorionic villus tissue showed methylation of genes controlling 24-hydroxylase enzymes (catabolic) while no methylation of vitamin D receptors in the placenta. The author concluded a possible epigenetic effect on the vitamin D

catabolism at the fetomaternal interface, functioning to maximize active vitamin D availability there (Novakovic, 2009).

A prospective cohort study in Turkey showed women with higher levels of 25(OH)D levels in their follicular fluid when undergoing an in vitro fertilization (IVF) cycle more likely to achieve clinical pregnancy (Ozkan, Jindal, Greenheid, Zeitlian, Hickmon, & Pal, 2009). When analyzing the results and adjusting for age, body mass index, ethnicity, and number of transferred embryos, each ng/mL increase in the 25(OH)D levels in the follicular fluid increased the likelihood of clinical pregnancy by 6%. Follicular levels and serum levels of 25-(OH)D were highly correlated ( $r=0.94$ ) (Ozkan et al., 2009).

### **Vitamin D Effects on Reproduction Eastern Viewpoint--General Perspectives**

Few references to vitamin D per se were found in the literature review. One interesting study was performed on forty-one British Bangladeshi individuals, aged 31-65, with the common south Asian addictive habit of chewing betel nuts (paan quid). Participants were questioned as to their cigarette smoking levels, betel nut usage, fish intake (especially the frequency of vitamin D rich fish), egg intake, and margarine consumption. Thirty six percent of the participants were men. VDR genotype was determined (ApaI, BsmI, TaqI, or FokI). Peripheral blood mononuclear cells (PBMC) were examined via polymerase chain reaction testing for levels of 1-alpha(OH)ase and 24(OH)ase mRNA and these results were related to serum 1,25 (OH)<sub>2</sub>D, 25-OHD, and parathyroid concentration levels. A direct correlation was found between 25-OHD-24(OH)ase expression and level of betel nut usage, regardless of VDR genotype. This is a curious and compelling finding as betel

nut chewing here is implicated in the induction of the 24(OH)ase gene in the presence of vitamin D that is not in excess. The induction of the 24(OH)ase gene is part of the mechanism ensuring vitamin D toxicity does not occur naturally due to sunlight exposure. The tissue production of 24(OH)ase is the rate limiting step in the degradation of 1,25(OH)<sub>2</sub>D—the enzyme is catabolic for both activated vitamin D and its precursor, 25-OHD (Ogunkolade, Boucher, Bustin, Burrin, Noonan, Mannan, & Hitman, 2006). In the *Materia Medica* for TCM, betel nut or Areca catechu is used in one of two forms: da fu pi (the husk of the betel nut) or bing lang (the seed). The TCM properties of da fu pi are to direct Qi downwards, expand the middle jiao, promote diuresis and relieve edema. Hence this form is used in formulas to treat such conditions as edema (especially of the legs) and feelings of distention and fullness in the epigastrium and abdomen or feelings of incompleteness after a bowel movement. (Chen & Chen, 2001; Bensky & Gamble, 1993). Bing lang is used to kill parasites (most common usage), promote qi movement and urination, dispel stagnation, and relieve nausea related to herbal prescriptions for conditions such as malaria (Bensky & Gamble, 1993; Chen & Chen, 2001). Both are recommended for use *with caution* in pregnancy due to known teratogenic and fetotoxic effects in mice (Bensky & Gamble, 1993; Chen & Chen, 2001). Habitual use of chewing bing lang is commonplace in Asia, and this habit causes a decrease in taste sensation, an increase in appetite, periodontal disease and oral discoloration (Bensky & Gamble, 1993).

The only herb referenced to contain vitamin D as a major chemical constituent for this study was bie jia. Bie jia, or *carapax amydae sinensis* (Chinese

soft-shelled turtle-dorsal aspect) is utilized within formulas to nourish yin and anchor the yang, invigorate the blood and soften hardness/dissipate nodules. Its use is contraindicated in pregnancy due to its dispersive and dissipating actions. Many other herbs have calcium, calcium salts, magnesium, phosphorus, or hormones of various types, such as zi he che (placenta), lu rong, mu li, long gu, gui ban, lu jiao, etc., but no reference could be found for their composition containing vitamin D or its analogues (Bensky & Gamble, 1993; Chen & Chen, 2001).

### **Dietary Considerations**

According to Flaws, the TCM function of vitamin D is to supplement the Kidneys and invigorate Yang, strengthen the sinews and bones, brighten the eyes and quiet the fetus (Flaws, 1998). Leggett believes vitamin D strengthens the bones, tonifies the kidneys and strengthens the jing or essence (Leggett, 1999). Individuals will often want to change their diet according to their geographical location if they are in tune with the natural surroundings. A connection exists between the foods native to a particular region and the people living there—plants and animals that have thrived in a region by utilizing available resources which differ from place to place—and this changes with the seasons. Metabolism changes from season to season, decreasing in winter in response to a colder external environment (David, 1991). The desire to ingest more protein and fats would generally traditionally provide more foods likely to contain vitamin D. Human beings have begun to eat more from a psychological place than a physiological place as traditional diets become less commonly ascribed to (David, 1991).

**Lifestyle Factors**

Traditional lifestyles advocated in the classics in TCM recommended living in peaceful locations with plenty of fresh air, sunshine, ventilation, sensible humidity, and a minimum of overstimulation from noise, pollution and the like (Flaws, 1998). Where one lives should be harmonious with nature.

**Literature Review Integration**

Research into vitamin D and its effects on fertility and gestational development from the allopathic medical perspective yielded significantly more research studies and articles than in the complementary medical perspective. This observation may be reflective of the relatively more modern concept of vitamin D as a named substance per se in medicine, and in the overall lack of high quality research studies performed to date on Traditional Chinese Medicine. More research is needed to further synthesize the literature that has emerged from both Western Medicine and Traditional Chinese Medicine regarding the relationship between vitamin D and fertility or gestational development issues. The objective of the current study is to contribute to that research path.



## Chapter Three: Method

### Research Method Designation—Statement of Methodology

The current study utilized grounded theory method. As a qualitative research method, grounded theory is effective in uncovering potential connections and identifying factors that may be followed up by additional and more controlled research studies (Bowen, 2006).

### Instruments: Definition of Grounded Theory

The grounded theory method of research provides a systematic means for information analysis and has been used extensively in the social science and nursing research arenas (Chiovitti & Piran, 2003; Weed, 2005; Pandit, 1996). The basis of a grounded theory research synthesis is the lack of hypothesis at the outset of data collection/generation. Inductive analysis allows theories to emerge from the analyzed data utilizing and interplay between the collection of the data and its analysis (Bowen, 2006). The general flow of these integrated processes are depicted in the following Table:

Table 3: Grounded Theory Data Collection Flow Chart

- ➔ Data Analysis → Theory Development → Theory saturation?
- ➔ If yes: you have reached closure
- ➔ If No: Repeat theoretical sampling → Data Collection → Data Ordering (coding) → Data analysis → Theory Development → Theory saturation?
- ➔ This cycle is continued till the answer is yes, at which point you have completed your data to be collected and made any inductive or logical analyses that can be made for the extant literature. (Pandit, 1996)

Grounded theory emerged as a research method in the last few decades, and has gained respect as a method for qualitative research. An area of inquiry is put forth and data gathered via various methods generally including books, research studies, and interviews/email exchanges. An open mind is kept during the data collection and coding of what is found performed to group ideas and themes that emerge in the process. Largely, this is an intuitive method to arrive at an understanding of what problems or values are present with regards to a topic or problem area. Reference lists from searched articles, leads from verbal interview discourse, etc. are followed up on and subsequent information obtained. This process is continued until all new avenues or leads are exhausted. When this occurs, the data groups are further coded into themes, and those themes into larger categories. The names for these types of codes are open coding (the labeling and categorizing of data elements), axial coding (the development of main categories and their subcategories) and selective coding (the integration of the categories developed from the two previous coding events) (Pandit, 1996).

### **Procedures**

For the current study, research articles were obtained by performing literature reviews in the following search engines: Google, Google Scholar, Pubmed, Medline, Liebert online, American Society of Reproductive Medicine (ASRM), and Ebsco host. Word of mouth leads for resources or knowledgeable sources for interview were contacted as they emerged. General information was obtained from scanning indexes of books in my extensive clinical library on human reproduction and Traditional Chinese Medicine. Search terms included: vitamin D, vitamin D

deficiency, vitamin D deficiency and fertility, vitamin D deficiency and human reproduction, vitamin D and Chinese medicine, vitamin D and Chinese herbs, light metabolism, Yin/Yang transformation, TCM differential diagnosis, and Kidney Yin/Yang. Articles found were printed, read and a recipe card summary made of the most salient points in the work. Each work was placed into a Summary Matrix Table (Appendix D) each theme resulting from the article summary being noted in the list of themes in the table. These recipe cards were then physically utilized to form groups or categories according to common themes and given a corresponding code; this step is contained in the “Open Coding For Each Theme” attached in Appendix A. Once this was accomplished, the groups formed from were then further sorted according to broader themes emergent in the codes. These were then given the coding designations of the axial coding phase, shown in “Axial Coding For Open Coding Categories”, Appendix B.

The final step required looking at these broader groups of research articles and their common themes, and from these deducing any apparent generalities that can be inferred about the influence of vitamin D deficiency in fertility and gestational development from the Western medical and the Eastern medical viewpoint. This is shown in the “Selective Coding Based on Axial Coding Western/Eastern” chart, also attached in Appendix C.

## Chapter Four: Results

### Data Analysis/Overview

Research articles collected for this study were categorized and coded in accordance with coding categories previously described in the Methods section for grounded theory analysis. The coding sequences inherently lend themselves to thematic analysis -- themes emerge from the data collection in an ongoing and inductive fashion, yielding an interpretation at the end of the dynamic process. Open coding is process of initial labeling and categorizing of data elements obtained from a summarization of the chosen research articles. Appendix A (refer to appendices) shows the initial categorization of articles according to general emergent themes of each article found. Appendix B takes the general individual article themes from Appendix A and consolidates the thematic groupings to further categories a few degrees broader in nature. This process, further extended in the next step, selective coding, is an inherent method within the grounded theory analysis namely inductively finding themes in the literature and current research that supports a hypothesis or hypotheses. Selective coding, the grouping of the axial coding groups into further related themes, are reported in Appendix C, see attached.

Data were analyzed using univariate statistics and thematic content analysis. Independent variables for the research articles obtained via database and reference list searches included the following:

- Vitamin D Serum level Insufficiency in Women of Childbearing Age

- Eastern/Chinese Medicine Perspectives on Vitamin D serum Level Insufficiency in Differential Diagnosis for and Gestational Development
- Western Medicine Perspectives on Serum Vitamin D Insufficiency levels for Differential Diagnosis and Gestational Development

Independent variables to be gleaned from the articles are the following:

- Effect on Fertility/Fecundity Levels
- Effect on Gestational Development
- Effect on General Health and Population

Simple counts were performed to produce tables for all research articles found.

**Table 4: Number of Articles/Information Sources Addressing The Dependent and Independent Variables**

	Effect on Fertility /pregnancy	Effect on Gestational Development	General/other Health Effects
Vitamin D Insufficiency in Childbearing Age Women	3	1	3
Eastern Medicine Perspectives			3
Western Medical Perspectives	19	7	24
Counts	<hr/> 22	<hr/> 8	<hr/> 30

General Information regarding the differential diagnostic characteristics of Vitamin D insufficiency is displayed in Table 5:

**Table 5: Signs and Symptoms of Vitamin D Insufficiency**

<b>Physiologic Location</b>	<b>Vitamin D and Metabolites Effects</b>
Intestine	Enhanced calcium transport
Bone	Mobilizes calcium from bone to fluid compartment, stimulates bone remodeling
Muscle	Maintains Integrity
Parathyroid glands	Inhibits Parathyroid hormone secretion
Immune System	Antitumor activity

Source: The Merck Manual of Diagnosis and Therapy, 1992

Signs and symptoms of vitamin D deficiency or insufficiency according to Western medicine can be compared to differential diagnoses used within the TCM framework to translate between the two medical philosophies of practice. By exploring the categories of diagnoses in TCM and the known influences of vitamin D on physiological symptoms in Western medicine, a broader understanding of differing nomenclature and alternative ways of approaching vitamin D deficiency effects in fertility and gestational development may be elucidated. Table 6 displays known western medical conditions which can be due to vitamin D deficiency or insufficiency, and shows the possible TCM diagnoses which could encompass those symptoms. Differences in nomenclature may or may not reflect differences in

treatment outcomes; further research studies will need to be designed to address these specifics.

**Table 6: Vitamin D Insufficiency In Eastern and Western Medicine**

Western Medicine signs and symptoms	Possible Differential Diagnoses  Traditional Chinese Medicine
Muscle Pain	Dampness with/out Spleen Qi deficiency (XU), may have heat, Liver Blood Xu, Qi Xu with Blood stasis or Blood Xu, Kidney Yang with/out Spleen Yang Xu
Fatigue/Low Energy	Spleen Qi or Spleen Yang Xu, Lung Qi or Lung Yang Xu, Kidney Yin or Yang Xu, Liver Blood Xu, Damp and/or Phlegm accumulation, Liver Qi stagnation
Lowered Immunity	Lung Qi Xu with/out Yin Xu or Heart Qi Xu
Depression/ Moodswings	Spleen/Heart Yang Xu, Spleen/Heart Blood Xu, Heart Blood or Yang Xu, Liver Blood Xu, Kidney/Heart Yin Xu with/out Empty Heat, Kidney Yang Xu, Liver Qi stagnation with/out Heat or Phlegm, Heart Blood stasis, Gallbladder Heat
Sleep Irregularities	Heart Blood or Yin Xu, Liver Yin or Blood Xu, Heart/Kidney Yin Xu with/out Empty Heat, Spleen/ Heart Qi and Blood Xu, Gallbladder Xu, Liver Fire, Phlegm Fire harassing the Heart
Weak Bones/Pain	Qi Xu with Blood stasis, Bi Syndrome, Kidney Yin, Yang, Qi or Essence Xu, Cold Invasion with/out Damp or Damp Heat

(Maciocia, 2004)

Number of information sources in each coding category are displayed in Table 7. Univariate statistics (descriptive analysis) using simple counts were performed on the totals obtained, and thematic analysis performed—the results will be compared and contrasted descriptively. Grounded theory is an inclusive method of data gathering, thus some articles were more directly pertinent to the variables of inquiry than others.

**Table 7: Open Coding Results of Vitamin D Insufficiency Influences On Fertility and Gestational Development**

Vitamin D Insufficiency and:	Enhancement/No Effect/(Not Differentiated)	Impediment Effect
Calcium Metabolism		2
Immunological Function	1	10
Pregnancy Loss		1
Fertility Rates		5
Gestational Development		3
Offspring Effects		2
Traditional Chinese Medicine	(2)	
Chinese Herbal Medicine	(3)	



Latitude/Geography		2
Seasonal Effects		2
Modern Diet		3
Traditional Diet		2
Cultural Effects		1
Light Metabolism		
Yin/Yang Symptoms		
General Health Effects		8
Hormonal Effects		4
Assisted Reproductive Technologies		2
Human Reproduction		7
(Total Articles)	(6)	(54)

### **Vitamin D and Fertility—Western Perspective**

The results of this literature synthesis revealed the following as one overall theme: insufficient serum levels of activated vitamin D appear to have adverse effects on general health and fertility.

### **Vitamin D and Gestational Development—Western Perspective**

The data from this literature synthesis also indicated insufficient serum levels of activated vitamin D in a pregnancy appear to have an adverse effect on human development and morbidity later in life.

**Vitamin D and Fertility—Eastern Perspective**

Current research databases have minimal data to directly indicate an effect on fertility from the TCM framework of diagnosis and treatment. However, when translated into TCM terminology and pathology patterns, physiological influences may be present. General information only was found in the data search.

**Vitamin D and Gestational Development—Eastern Perspective**

Similar to the above statements, no direct link between vitamin D serum levels and TCM diagnoses related to gestational development and pregnancy pathologies were found during the literature synthesis process. Effects may be understood from a translational viewpoint between the functions of vitamin D known from evidence based medicine, correlated with TCM diagnostic signs and symptoms. The paradigms of TCM are not in conflict with the western medical research.

## **Chapter Five: Discussion**

### **Summary of Findings**

Published research articles concerning the effects of Vitamin D on general health or discussing its mechanisms of action as we know it today are plentiful in the literature. Less numerous, but increasingly represented in the most recent research Western medical literature are inquiries into the effects this prohormone has on reproduction and gestational development. For all intents and purposes, no research articles addressing the influence of vitamin D on reproduction and gestational development were found from the eastern medical perspective. However, discussions from Classical Chinese Medicine sources contain relevancy to the topic at hand.

### **Implications For Theory**

Western medical research fairly overwhelmingly points to a regulatory role for vitamin D in pregnancy and gestational development, whether a negative influence in the case of fertility and gestation due to insufficient/deficient levels, or from elevated levels potentially adversely affecting the development of endometriosis or impaired regulation of the immune modulation at the placenta interface.

In the arena of Traditional Chinese Medicine, a broader interpretation of classically held truths in the practice of the medicine may be possible in light of the results from current research. In the TCM philosophy of fertility and reproduction, the Jing of the parents is a major determinant of the reproductive status of their offspring. The age of both parents, their constitutional makeup, overall health and

the conditions of any pregnancy help determine the inherited Jing or constitutional makeup of their children (Maciocia, 1994). This inherited deficiency may be exhibited in any of the five Yin organs: Heart, Lungs, Spleen, Liver, or Kidneys (Maciocia, 1994). The diagnostic cause for over half the cases of infertility according to TCM is believed to be Kidney Yin, Yang, or Qi related (Maciaocia, 2004); Kidney Yang Xu (deficiency) is ascribed to as many as 80% of infertility patients according to Yaron Siedman, founder of the Hunyuan Method (Seidman, 2009). Kidney energy in all its permutations (Qi, Yin, Yang, Essence) is associated with the bones, the reproductive system, the strength of the will, and the regulation of development. It is the source of the Ming Men Fire, the spark of life itself (Maciocia, 1989). One of the classics of TCM, *The Yellow Emperor*, states that one's lifestyle must follow the sunlight in winter or the lifespan will be shortened (Seidman, 2009). The ancients had an appreciation of the need to be in rhythm with the natural cycles, which connects our external environment to our internal environment or health. In modern times usually no alteration of our day to day lifestyle is made with regards to the changing energies of the seasons—Americans, in general, value doing or Yang activities at the expense of the Yin. Vitamin D has usually been ascribed to a Kidney Yang function mostly with regards to the regulation of calcium metabolism, affecting the control of Kidney Yin (calcium is a yin substance). By being out of synch with the natural Yang of sunlight, this researcher wonders if that causes a bigger draw on the individual's own internal Ming Men Fire, resulting in premature aging, immune system inefficiency, infertility, and the overall symptoms associated with vitamin D

insufficiency. Thus, a serum vitamin D level may come to be utilized as a marker of relative harmony and connection with the natural world.

When considering the impact of vitamin D in the epigenetic control of gene expression at the fetomaternal interface, correspondences with the traditional view of Lung /Wei Qi appear to be present. Wei Qi or Defensive Qi is part of the expression of Lung Qi, and as such is associated with the surface of the body or the skin (Seidman, 2009). The lining of the uterus could be considered to be a body surface that correlates to the skin, albeit internally protected. Immune system regulation at the endometrial level with implantation makes sense from a Wei Qi standpoint in such conditions as autoimmune recurrent pregnancy loss, antiphospholipid antibodies, or other autoimmune conditions possibly influenced by vitamin D levels and the individual's inherited VDR status. Fetal education, a set of rules ancient Chinese gynecologists, appears to encompass what could be epigenetic influences on the gestational development of a growing fetus in utero. The basis of Fetal Education rested on the diet, emotions, and lifestyle affecting the fetus. Rules to be followed varied month to month, corresponding to the differing physiological developments occurring in the baby. By following these rules, an expectant mother hoped to positively affect her child's constitution, temperament, anatomical and energetic development (Maciocia, 1998).

The two medical philosophies come from differing paradigms; however as more research is done from a Western medical perspective, a broader understanding of vitamin D's influences may be incorporated into the TCM view of its function such as the regulation of Wei Qi (highly associated with Lung

energetics), or with the regulation of Liver or Spleen diagnostic patterns (associated with digestive efficiency, absorption of nutrition, and activation of vitamin D in its metabolic pathway) in the TCM framework. This is a departure from current theory according to some experts in the field who state the Lung energy does not play an important role in gynecological issues (Maciocia, 1998). Others acknowledge the Lung's function in Governing Qi to have at least some influence in the fertility arena (Lyttleton, 2004).

### **Implications for The Practice**

Thematic analysis of existing research studies and inductive reasoning correlating the signs and symptoms of vitamin D insufficiency in childbearing age women may result in further research in the field to ascertain exact levels of supplementation require, for whom, with what types of diet or cultural norms and at what latitudes. Comparisons and parallels drawn from Eastern and Western Medicine viewpoints may foster information exchange and integration of practices between the two treatment philosophies. Clear guidelines for referral, supplementation recommendations, and ways of approaching human reproduction and gestational development may be augmented or spur further inquiry. And finally the complexities of the factors influencing maximal health and lifestyles may be elucidated to a further degree.

### **Limitations of the Current Study**

The grounded theory method has several potential limitations. Theoretical sampling is an iterative process in grounded theory. The scope of the research question for this inquiry is vast and universal database access by the principal

researcher impractical making exhaustive data collection unlikely. Insufficient data sampling, i.e. premature cessation of article retrieval, may result in an incompletely formed series of themes or conclusions related to the inquiry subject. While thematic analysis is somewhat inherent in the coding process utilized for grounded theory research, the concurrent data collection and analysis may reflect a bias due to incomplete information if any idea streams are not completely followed and article retrieval sources exhausted. Validity of the final result may be compromised. Internal bias may be present in the principal investigator's compilation of themes and analysis thereof may be present, particularly in an area of inquiry such as this research project where minimal research articles are available for one side of the inquiry question (research into vitamin D effects within the Traditional Chinese Medicine field). In the interpretation of this area the study takes on a more phenomenological analysis flavor than a frank grounded theory method.

### **Recommendations for Future Research**

Future research opportunities in this area of inquiry are numerous. Among the areas for further research are correlating vitamin D levels for infertility or pregnant women and their partners with their working TCM diagnoses, and following diagnostic changes as individuals with deficient levels are brought into insufficient and then replete status in their vitamin D levels. A longitudinal study looking at the offspring of vitamin D insufficient or deficient couples, their TCM and Western medical diagnoses over their lifespan and any correlations would also provide interesting baseline information. Another possibility would be to identify infertile women with insufficient levels of vitamin D, institute a lifestyle prescription

as put forth in the classics and analyze whether vitamin D levels or fertility status consequently shifted. Comparison of vitamin D levels and fertility rates in couples getting 30 minutes of unfiltered sunlight daily with or without acupuncture and/or herbal treatment may yield relevant information to affect standard of care for infertile couples in the future. Finally studies looking into the resolution of TCM Phlegm/Damp conditions (of which obesity is one manifestation, PCOS another) thru herbal therapy, lifestyle changes, and acupuncture and its impact on hydroxylase enzyme production and efficiency would be helpful to perform with an eye towards fertility enhancement in subfertile individuals.

Further research to analyze fertility rates in by latitudes, season and race with and without prenatal and gestational TCM treatment and lifestyle prescriptions also warrants investigation. This data should be correlated with the vitamin D levels of the mother and father in the prenatal, natal and postnatal stages. Analysis of herbal Kidney yang tonic formulas with respect to vitamin D levels in couples trying to conceive as well as any influences on gestational development and later life morbidity in offspring represents a further area for TCM research. An analysis of infertile versus fertile men and women and their relationship with the outdoor environment—attitudes, practice, beliefs, fears, etc.-- to establish a baseline correlation between vitamin D serum levels and degree of disconnection to natural rhythms and cycles could prove enlightening.

Herbal formula effects on enzyme production and activation rates in the vitamin D pathway, particularly at rate limiting steps, need to be investigated at the level of the TCM Kidney, Liver, Spleen and Stomach. Further the influences of TCM



herbal formula treatment on VDR binding capacity and efficiency should be ascertained both in vivo and in vitro.

From a western perspective future research is the need to establish correlations between NK assays and vitamin D levels for infertile or RPL women, then ascertain whether those assay results or rates of live birth are affected by blood and/or tissue vitamin D levels. Another series of studies could analyze vitamin D amounts bound to receptors in the ovary, testes and placenta and any relationship with infertility, RPL, maternal or infant morbidity or mortality. The inclusion of TCM treatments versus placebo on rates and relative levels of VDR and their binding capacity and efficiency may yield deeper understanding of the mechanisms of TCM influence within this system. Measurement of vitamin D and calcium levels in follicles of successful IVF cycles and whether or not more eggs fertilize if both the egg and sperm donor are at replete levels is another arena to explore, as well as looking into the effects of TCM treatments of acupuncture and/or herbal formulations on these levels. A final area for future investigation regarding Western Medicine is the generation of data regarding the genetic polymorphisms of VDRs present in reproductive tissue, corresponding rates of vitamin D binding and activation, and the potential influences of acupuncture treatment could impart on epigenetic up/down regulation of receptor expression, binding rates, binding efficiency and concomitant effects when TCM treatment is utilized versus when it is not.

## Conclusions

The above literature analysis clarifies the apparent lack of ability to translate treatment philosophies in the field of reproductive medicine between Western and Eastern Philosophies. While both medical approaches address the same end result, Eastern medicine does so in a broader fashion with an emphasis on the individual's relationship to his or her environment. When that relationship is reasonable, balanced and in harmony, men and women of childbearing age are considered to be fertile, barring any physical trauma or scarring to the reproductive tract. Vitamin D levels per se are not necessary pieces of information from this perspective. Harmony to the cycles of Yin and Yang are. A life lived according to the ancient recommendations naturally supports adequate sunlight exposure, dietary ingestion, and regulation of the vitamin D metabolism. The potential insufficiency can be recognized by lifestyle departures from established ideals and rectified by necessary changes in behavior.

However, in modern society, and especially in the arena of reproductive medicine, laboratory values are utilized to make diagnoses in western medicine far more often than by a constellation of symptoms. Infertility patients and pregnant women are diagnosed and monitored via objective measures, laboratory testing being one of the measures utilized. Overall optimum health is not immediate the focus—identification of individual pathology and its return to normal parameters are. More and more the two differing approaches are asked to work in an integrated fashion for the benefit of the fertility patient. Chinese medical practitioners utilize other laboratory test results such as FSH or P4 to monitor treatment progress and

influence lifestyle, diet and herbal recommendations. The current researcher posits knowledge of the vitamin D level needs to be included in standard of care for any infertile couple. Most humans have serum levels from sunlight exposure or dietary intake capable of preventing rickets. Levels must be dangerously low for rickets to occur. Levels of insufficiency however, result in humans living at below optimum health levels and that impacts fertility and quality of life in many subtle ways such as decreased immunity, depression, or musculoskeletal morbidity. For optimum fertility, all physiological systems must be operating well—evolutionarily fertility is not geared to occur in times of scarcity, when there is not enough to sustain the pregnancy or the life of the infant (Meletis, 2004). The impact of vitamin D insufficiency may well be of major importance in couples experiencing subfertility, and it is certainly one factor which can be rectified fairly readily and easily. The ability to mutually understand information and viewpoints in the area of vitamin D and its effects on fertility is just one arena where a commonality of understanding is beneficial for the patient.

Vitamin D levels and/or its Yin Yang counterpart influence optimum fertility and gestational development when in a replete state. Both Eastern and Western Medicine have ways to analyze the associated pathologies, the syntax of each system is different. In modern times, it is likely that the Western serum level will be used by western and eastern practitioners to influence standard of care in women with infertility and or gestational development issues. The treatment used to rectify any discovered insufficiencies in the future may be varied to include more traditional,

holistic approaches rather than mere supplementation as further research into the complex effects of vitamin D on homeostasis are further elucidated.

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## **Appendix A**



## Appendix A

## Open Coding Categories For Each Theme

	Replete = Positive Effect	Deficiency = Negative Effect
Vitamin D/calcium metabolism	1A	1B
Vitamin D/ immunological function	2A	2B
Vitamin D/recurrent pregnancy loss	3A	3B
Vitamin D/fertility	4A	4B
Vitamin D/gestational effects	5A	5B
Vitamin D/long term effects in offspring	6A	6B
Vitamin D/Traditional Chinese Medicine	7A	7B
Vitamin D/ Chinese Herbs	8A	8B
Vitamin D/Latitude	9A	9B
Vitamin D/seasonal effects	10A	10B
Vitamin D/ Modern American Diet	11A	11B
Vitamin D/Traditional Chinese Diet	12A	12B
Vitamin D deficiency symptoms	13A	13B
Solar UV intensity/effects on humans	14A	14B
Vitamin D/Cultural effects	15A	15B
Vitamin D/Light Metabolism	16A	16B
Kidney Yin/Yang Deficiency Symptoms	17A	17B
Vitamin D/general information	18A	18B
Vitamin D/Invitro Fertilization	19A	19B
Vitamin D/Assisted Reproductive Technologies	20A	20B
Vitamin D/Human reproduction	21A	21B

Number and letter designation (1A, 1B, etc.) identify groups of research articles with similar conclusions into open coding categories. These categories are one step beyond the Research Synthesis Matrix (Appendix D).

## **Appendix B**

## Appendix B

## Axial Coding For Open Coding Categories

Open Code	Axial Code AA	Axial Code BB	Axial Code CC	Axial Code DD	Axial Code EE
1A	AA				
2A	AA				
3A		BB			
4A		BB			
5A		BB			
6A		BB			
7A			CC		
8A			CC		
9A				DD	
10A				DD	
11A					EE
12A					EE
13A				DD	
14A				DD	
15A					EE
16A					EE
17A			CC		
18A				DD	
19A		BB			
20A		BB			
21A		BB			
1B	AA				
2B	AA				
3B		BB			
4B		BB			
5B		BB			
6B		BB			
7B			CC		
8B			CC		
9B				DD	
10B				DD	
11B					EE
12B					EE
13B				DD	
14B				DD	
15B					EE
16B					EE
17B			CC		
18B				DD	
19B		BB			
20B		BB			
21B		BB			

## Appendix B

Double letter designations (AA, BB, etc.) indicate groupings of similar research articles from the Open Coding categorization results from Appendix A.

I.e., research articles in Axial Coding group AA includes articles with a conclusion that Vitamin D has an effect on measured outcomes with respect to calcium metabolism (open coding category 1A and 1B) and immunological function (open coding category 2A and 2B) are now more generally grouped together.

## **Appendix C**

## Appendix C

## Selective Coding Based on Axial Coding Western/Eastern

Axial Coding Assignment	Western Medicine Role	Eastern Medicine Role
AA	W	
BB	W	E
CC		E
DD	W	E
EE	W	E

Letter designations (W or E) indicate groups of research articles relevant to vitamin D's influence on measured outcomes from a Western or Eastern perspective, and represents further generalized groupings of the gathered data from the Axial Coding categories.

## **Appendix D**







## Appendix D

THEMES	Authors											
	25	26	27	28	29	30	31	32	33	34	35	36
Vitamin D/Calcium Metabolism		X		X		XM			XM			
Vitamin D/ Immune Function		XM		X		X	XM			XM	XM	
Vitamin D/Pregnancy Loss					XM						X	
Vitamin D/Fertility		X			X							
Vitamin D/Gestational Effects	XM		X	X								
Vitamin D/Long Term Offspring Effects	X		XM	X								
Vitamin D/Traditional Chinese Medicine												
Vitamin D/ Chinese Herbs												
Vitamin D/Latitude												
Vitamin D/Seasonal Effects				XM								
Vitamin D/Modern Diet												
Vitamin D/Traditional Diet												
Vitamin D Deficiency Associations												X
UV intensity/Human Effect												
Vitamin D/Cultural Influences												
Vitamin D/Light Metabolism												
Kidney Yin/Yang Patterns												
Vitamin D/General Information						X	X					
Vitamin D/Assisted Reproductive Technologies												
Vitamin D/Human reproduction					X	X			X	X	X	XM
Vitamin D/In vitro Fertilization								XM				





## Key to Authors Associated with Numbers 1-53 in Appendix D:

NUMBER	AUTHOR/S	NUMBER	AUTHOR/S
1	Holick, 1995	31	Somigliana, et al 2007
2	Holick, 2007	32	Potashnik, et al 1992
3	Vieth, 1999	33	Jensen, et al 2011
4	Zitterman, 2003	34	Evans, et al 2006
5	Rajakumar, 2003	35	Diaz, et al 2009
6	Rajakumar, et al 2007	36	Murphy, et al 2010
7	Zeisel, 2000	37	Bakare, et al 2011
8	Holick, 2008	38	Grayson/Hewison 2011
9	Sellar, 2005	39	Stephensen 2011
10	Maetani, et al 2009	40	Meroni, et al 2009
11	Looker, et al 2008	41	Bodnar, et al 2009
12	Schaefer 1959	42	Hensel, et al 2011
13	Kuhnlein, et al 2007	43	Mahon, et al 2010
14	Nesby-O'Dell, et al 2002	44	Mulligan, et al 2010
15	Ginde, et al 2010	45	Novakovic 2009
16	Johnson, et al 2010	46	Ogunkolade, et al 2006
17	Pilz, et al 2010	47	Chen/Chen 2001
18	Kwiecinski, et al 1989	48	Bensky/Gamble 1993
19	Halloran/Deluca 1980	49	Flaws 1998
20	Kinuta, et al 2000	50	Leggett 1999
21	Zanatta, et al 2011	51	David 1991
22	Ahonen, et al 2000	52	Ozkan, et al 2009
23	Yoshizawa, et al 1997	53	Eliot 1926
24	Levenson/Figueroa 2008	54	Palm 1890
25	Eyles, et al 2009	55	Natl Research Council 1989
26	Panda, et al 1992	56	Martini/Wood 2006
27	Gezmish, et al 2010	57	Holick 2011
28	Lucas, et al 2008	58	Webb/Holick 1988
29	Evans, et al 2004	59	Will, et al 2009
30	Jones, et al 1998	60	Reddy, et al 1983

## Legend:

X = research article addresses this theme

XM = main category or coding