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Replete vitamin D stores predict reproductive success following IVF

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Abstract

Objective—Hypothesizing that levels of 25OH-D in body fluids are reflective of vitamin repletion status, we aimed to determine if 25OH-D levels in the follicular fluid (FF) of infertile women undergoing in vitro fertilization (IVF) demonstrate a relationship with IVF cycle parameters and outcome.

Design—Prospective Cohort study

Setting—Academic tertiary care center

Patients—84 infertile women undergoing IVF

Interventions—FF from follicles ≥14mm; serum (n=10) and FF levels of 25OH-D

Main outcome measures—Clinical pregnancy (CP) (defined as evidence of intrauterine gestation sac on ultrasound) following IVF; IVF cycle parameters.

Results—Serum and FF levels of 25OH-D were highly correlated (r=0.94, p<0.001). In a predominantly Caucasian population (66%), significantly lower FF 25OH-D levels were noted in Black versus non-Black patients (p=0.001). Significant inverse correlations were seen between FF 25OH-D levels and BMI (r−0.25, p=0.035). Significantly higher CP and implantation rates were observed across tertiles of FF25OH-D (p=0.029 and p=0.041 respectively); patients achieving CP following IVF (n=26) exhibited significantly higher FF levels of 25OH-D (p=0.005). Multivariable logistic regression analysis confirmed FF 25OH-D levels as an independent predictor to success of an IVF cycle; adjusting for age, BMI, ethnicity and number of embryos transferred (ET) each ng/ml increase in FF 25OH-D increased the likelihood for achieving CP by 6% (p=0.030).

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Conclusion—Our findings that women with higher vitamin D level in their serum and FF are significantly more likely to achieve CP following IVF-ET are novel. A potential for benefit of vitamin D supplementation on treatment success in infertile patients undergoing IVF is suggested that merits further investigation.

Keywords

Vitamin D; 25OH-D; infertility; in vitro fertilization; Clinical Pregnancy; Follicular Fluid

INTRODUCTION

Vitamin D, a steroid hormone, is well known to be involved in Calcium-Phosphate (Ca-P) homeostasis and bone metabolism (1,2,3). Emerging data identify critical roles for vitamin D in a variety of other biological processes including regulation of cellular growth and differentiation and metabolic modulations specifically involving insulin action (4 and references therein). Indeed, beneficial roles for vitamin D in a spectrum of pathological processes including autoimmunity, insulin resistance, cardiovascular disease and malignancies are emerging concomitant with the appreciation of a global *pandemic* of vitamin D deficiency (4–5). Those biological actions are mediated through vitamin D Receptor (VDR) (3,6). VDR is a member of the steroid/thyroid nuclear hormone receptor superfamily and has been demonstrated in calcium regulating tissues; intestines, skeleton, parathyroid glands as well as reproductive tissues including ovary, uterus, placenta, testis and the hypophysis (7–10).

Amongst the many physiological processes impacted upon, critical roles for vitamin D in reproductive physiology are suggested (1,8–15). Experiments investigating the significance of vitamin D for fertility and reproductive capacity, while demonstrating that the vitamin may not be critical for successful female reproduction, do demonstrate compromised mating behavior, reduced fertility rates, decreased litter sizes and impaired neonatal growth in vitamin D deficient female rats (1). Similar evidence of reproductive compromise in male rats deficient in vitamin D has been identified (11). Data on implications of vitamin D on reproductive physiology in non-pregnant subjects are limited to a few experimental investigations in animal models; specific human data in this context are sparse. While able to reproduce, vitamin D deficient rats demonstrate diminished mating success and fertility capacity (1); reduced litter sizes and impaired neonatal growth are also described as is an overall reduction in fertility by 75%, primarily attributed to decreased mating rates and increased pregnancy complications (1). Expression of VDR in reproductive tissues and a hamster ovarian cell line further pointed out a potential role of vitamin D in female reproduction (6).Vitamin D has been shown to promote Ca transport in placenta, stimulate lactogen expression, decidualization of endometrium and regulate HOXA 10 expression (a target gene related with implantation process) contributing to data related with vitamin D-reproduction (4,16–18). Additional experimental studies with VDR null mutant mouse models demonstrated gonadal insufficiency, reduced aromatase gene expression, low aromatase activity, hypergonadotropic hypogonadism (7) and features of estrogen deficiency such as bone malformations, uterine hypoplasia, impaired folliculogenesis and infertility (3). Vitamin D has thus been identified as mandatory for reproductive function at least in the murine model (12).

While alterations in calcium-phosphate metabolism are suggested to partly explain the reproductive sequelae of vitamin D deficiency, adverse implications for ovarian steroidogenesis and uterine receptivity are also described (3,6,11,14–15). Some investigators have attributed the reduced fertility rates seen in vitamin D deficient animals as direct effect of vitamin D rather than the hypocalcemia associated with D deficiency. Others however suggest that at least in male rats, fertility is most critically affected by calcium levels, independent of vitamin D; indeed calcium has been shown to affect sperm maturation,

capacitation and acrosome reaction (15), and in vitamin D deficient rats, normalization of reproductive capacity has been reported by feeding a high Ca and P diet alone (14). Yet others have demonstrated that consumption of a vitamin D deficient diet prior to and during pregnancy in rats adversely affects fecundity rates independent of female age and BMI.

Available literature is supportive of roles for vitamin D in placental steroidogenesis, calcium transport through the placenta, expression of placental lactogen and decidualization of endometrium; additionally, vitamin D has been identified to regulate key target genes related with implantation and in establishment of the fetoplacental unit (9,16,17,19). Decreased aromatase activity and reduced aromatase gene expression in the ovary, testes and epididymis of animals deficient in vitamin D are described (7). The available data thus identify vitamin D as a key player in processes involved in reproductive success and thereby suggest pathophysiological mechanisms for reproductive compromise in the setting of vitamin D deficiency.

Realizing the pandemic of vitamin D insufficiency, in the setting of an increasing appreciation of vitamin D's myriad roles in health in general, and in reproductive physiology in particular, we herein hypothesized that replete vitamin D stores will translate to improved reproductive success following in vitro fertilization (IVF). Given the proximity of the developing oocytes to the follicular fluid (FF), we further hypothesized that higher FF 25OH-D levels will be associated with improved ovarian response to controlled ovarian hyperstimulation (COH).

METHODS

A prospective cohort study was undertaken at the Montefiore Institute for Reproductive Medicine and Health. Eighty four infertile women undergoing IVF were enrolled between March 2005 - December 2007. The study protocol was in accordance with the guidelines of Declaration of Helsinki, was approved by Institutional Review Board (IRB) at the Montefiore Medical Center and the participants provided written consent.

All patients enrolled in the study underwent IVF cycles as per standard clinical care. Standardized regimens for controlled ovarian hyperstimulation (COH), pituitary down regulation, Leuprolide Acetate [Lupron®; TAP Pharmaceuticals North Chicago, IL, USA], starting in the midluteal phase at a dose of 0.5mg/day SC or Ganirelix Acetate [Antagon™, Organon Inc. West Orange, NJ] started following initiation of COH with E_2 levels reaching ≥400pg/ml or dominant follicle size ≥14mm), and ovulation triggering were instituted. COH was initiated with recombinant FSH and starting dose was selected on the basis of age, early follicular FSH levels and the number of antral follicles; individualized step-down or step-up protocols were instituted and serial monitoring of ovarian response assessed by transvaginal ultrasound and serum estradiol (E_2) assays. Nuclear maturation was triggered with 10, 000 IU hCG intramuscularly when 3 or more follicles >17 mm were achieved. Serum samples were collected on the day of hCG administration (approximately 34 hours prior to egg retrieval) and stored at −20° C until assayed.

Transvaginal ultrasound guided oocyte retrieval was performed 34 hours following the hCG injection. Follicular fluid (FF) was collected from follicles ≥14mm; following oocyte isolation, FF for each patient was pooled, centrifuged at 3000g for 15 minutes and the supernatant was stored at −80°C until assayed. Fertilization was assessed 17–18 hours post insemination. Ultrasound guided fresh embryo transfer (ET) was carried out on day 3 post insemination. The luteal phase was supported by intramuscular Progesterone (P) in oil (50mg/day); positive serum hCG tested 12 days after embryo transfer was considered as evidence of implantation and P supplementation was continued until documentation of fetal cardiac activity. Clinical pregnancy (CP) was defined as intrauterine gestational sac visible on transvaginal ultrasound.

Patient and cycle parameters were identified from clinical records including age, self-reported ethnicity, infertility etiology, infertility duration, body mass index (BMI), early follicular phase hormonal assessment of ovarian reserve (FSH and E_2), IVF cycle stimulation protocol (i.e. duration of stimulation in days, total FSH ampoules used for COH), number of ovarian follicles >14 mm on day of hCG, serum E_2 and P levels on day of hCG, total oocytes retrieved and number of mature oocytes, fertilization rate, number and cleavage status of ET. Outcomes of interest were implantation rate (number of gestational sacs identified on ultrasound / number of ET × 100) and CP following fresh ET.

Based on previously defined serum criteria (5), FF 25OH-D level >30ng/ml was defined to reflect "replete" vitamin D status; level between 20–30ng/ml was taken to reflect vitamin D insufficiency whereas 25OHD<20ng/ml defined evidence of Vitamin D deficiency.

STATISTICAL ANALYSIS

Continuous data are reported as mean ± standard deviation (SD) and categorical as percentage (%). Univariate analyses determined associations between FF 25OH-D levels with patient and cycle parameters (Student's t test, Mann-Whitney U test as appropriate). Tertiles of FF 25OHD were computed (25OH D levels from lowest to highest tertiles were 16.74 ±3.38, 25.58 ± 3.17 and 43.01±10.65 ng/dl respectively); proportion of patients achieving CP and implantation rate across the tertiles of 25OHD distribution were assessed by Kruskall Wallis Rank Test. Multivariable logistic regression analysis evaluated the relationship between FF 25OH-D and CP after adjusting for parameters known to influence success of an IVF cycle (age, BMI, ethnicity and number of ET). Likelihood of CP is presented as odds ratio (OR) ± 95% confidence interval (95% CI). Because of the relatively small dataset and the few outcomes (i.e. CP), a propensity score analysis was employed (20). The propensity score, derived from a separate multivariate logistic model incorporating the adjustment co-variates, was utilized as a single adjustment variable (summarizing the covariates) within the logistic regression models determining an association between CP and FF 25OHD levels. STATA IC 10 (StataCorp, College Station, TX USA) was used for the statistical analysis. $P<0.05$ was considered to be statistically significant.

RESULTS

Serum and FF levels of 25 OH vitamin D were highly correlated ($r=0.94$, $p<0.001$, Figure 1) demonstrating that FF levels of 25OH-D indeed were reliable reflectors of body stores of the vitamin. As per serum level criteria (5), only 37% of participants demonstrated replete 25OH-D stores (>30ng/ml); 36% met criteria for insufficiency (20–30ng/ml) whereas 27% were vitamin D deficient (<20ng/ml) (Figure 2).

In a predominantly Caucasian population (66%), significantly lower FF 25OH-D levels were noted in Blacks (n=12) compared to patients of non Black ethnicity (n=64) (18.88 ± 8.5ng/ml versus 30.51± 12.95 ng/ml, p=0.001). Significant inverse correlations were noted between FF 25OH-D levels and BMI ($r=-0.25$, $p=0.035$). Although lower FF 25OHD levels were noted in eight patients with polycystic ovarian syndrome (PCOS) and in twenty women with diminished ovarian reserve (DOR) compared to those with other infertility etiologies (26.19 ±11.22 in PCOS versus 28.57±13.09 ng/ml in non-PCOS patients and 24.16 ± 9.57 in DOR versus 29.65±13.5 ng/ml in patients with normal ovarian reserve), these differences were not of statistical significance ($p>0.05$).

Table 1 describes the participant and IVF cycle characteristics by outcome of IVF cycle (i.e. CP versus not pregnant). Those achieving CP (n=26, 30.95%) demonstrated significantly higher FF 25OH-D compared to those with unsuccessful cycles (n=58, 69.04 %, p=0.013, Figure 3), utilized significantly lesser doses of gonadotropin ampoules for shorter duration (in

days) and were transferred significantly higher number of embryos compared to those whose IVF cycles were unsuccessful; the remaining IVF cycle characteristics did not differ significantly (Table 1) and the proportions of cycles utilizing GnRH agonist versus antagonist were similarly comparable across the two groups (in those achieving CP, 55% of cycles utilized GnRH agonist compared to 53% in those with failed outcome, p=0.857).

While significant differences were observed in cycle parameters in patients achieving CP and those with failed outcomes as specified in Table 1, no direct relationship was observed between patient and cycle parameters and 25OH-D levels, i.e. there was no correlation observed between FF 25OH-D levels and ovarian response parameters (i.e. duration of COH, number of follicles, number of eggs retrieved, maximal estradiol) nor with ovarian reserve parameters (age or FSH) (data not shown). Although a trend towards increasing ovarian response (shorter duration of COH, increasing estradiol levels) was observed across tertiles for vitamin D, these differences were not statistically significant (p>0.05, data not shown).

Significant increase in implantation and CP rates was observed across tertiles of 25OH D distribution (Figure 4, p=0.041 and 0.029 respectively); all other patient and cycle parameters were comparable across tertiles of 25OH D including patient age, BMI, FSH, dose of gonadotropins, duration of COH, maximal estradiol, number of eggs retrieved and number of embryos transferred (data not shown). Those in the highest tertile for 25OH D were almost four times more likely to achieve CP compared to patients in the lowest tertile (OR for CP 3.83, 95% CI 1.20–12.28, p=0.024).

Multivariable logistic regression incorporating propensity score analyses confirmed FF 25OH-D levels as an independent predictor of success of an IVF cycle; adjusting for age, BMI, ethnicity and number of embryos transferred, each ng/ml increase in FF 25OH-D level enhanced the likelihood for achieving CP by 6% (p=0.030, Table 2). Alternatively, those with FF 25OHD levels in the lowest to mid tertiles were 75% less likely to achieve CP compared to women with FF Vitamin D levels in the highest tertile (OR for CP 0.25, 95% CI 0.07–0.84, p=0.026).

Of the 84 IVF cycles, 81 were first ART cycles whereas 3 were repeat cycles. Sensitivity analyses were conducted excluding the 3 repeat ART cycles and confirmed essentially unchanged magnitudes of association, (OR's, CI's and p values) in the previously observed relationships between 25OHD and IVF cycle outcome (data not shown).

DISCUSSION

We herein demonstrate that FF levels of 25OH vitamin D are reflective of body stores of vitamin D. While our study is limited by the relatively small sample size, our findings of significantly higher FF levels of vitamin D in those achieving clinical pregnancy following IVF are indeed novel, not previously described and may hold potential therapeutic implications. Improved COH parameters (albeit insignificantly so) in the context of higher vitamin D levels are suggestive of facilitatory implications of FF 25OHD on ovarian steroidogenesis; these observations are hence in keeping with published literature (3,7,8).However, in the absence of any significant relationship with ovarian response, our observations may identify endometrial receptivity as the potential target for beneficial influences of higher circulating vitamin D levels. Vitamin D has been previously identified as a regulator of endometrial expression of HOXA10, a target gene critical to implantation process (18), and our observations could thus be explained by this proposed relationship.

The magnitude of prevalent vitamin D insufficiency and the ethnic disparity in status of vitamin D repletion as seen in our data are consistent with prior reports. The prevalence of vitamin D insufficiency/deficiency in various communities is reportedly staggering (6) and our findings

corroborate this impression. Socio-economic disparity is a recognized contributor to nutritional deficiencies across populations, and is also well identified as a hurdle for access to infertility care. Our findings thus suggest nuances other than socio-economic inequality, for example lifestyle, that may be contributory to insufficient vitamin D stores in a population of otherwise healthy infertile women undergoing IVF (and hence deemed to be economically sound).

The observed prevalence of vitamin D insufficiency in otherwise healthy population is concerning especially in the context of accruing data on beneficial influences of replete vitamin D stores on multiple physiological processes, and emerging roles of vitamin D insufficiency in a spectrum of diseases. Given our findings, assessment of vitamin D status may be entertained as a part of routine infertility workup as appropriate supplementation of those deemed deplete in vitamin D *may* translate to improved fertility outcome as well as improved overall health. These latter conjectures merit further assessment by appropriately designed longitudinal studies.

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REFERENCES

1. Halloran BP, DeLuca HF. Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. J Nutr 1980;110(8):1573–1580. [PubMed: 7400847]
2. Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN, et al. Targeted ablation of the 25-hydroxyvitamin D 1 alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proc Natl Acad Sci U S A 2001;98(13):7498–7503. [PubMed: 11416220]
3. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet 1997;16(4):391–396. [PubMed: 9241280]
4. Heaney RP. Vitamin D in health and disease. Clin J Am Soc Nephrol 2008 Sep;3(5):1535–1541. Epub 2008 Jun 4. [PubMed: 18525006]
5. Holick MF. Vitamin D Deficiency: Review. N Engl J Med 2007;357:266–281. [PubMed: 17634462]
6. Johnson LE, DeLuca HF. Vitamin D receptor null mutant mice fed high levels of calcium are fertile. J Nutr 2001;131(6):1787–1791. [PubMed: 11385068]
7. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinol 2000;141:1317–1324.
8. Hurley WL, Doane RM. Recent developments in the roles of vitamins and minerals in reproduction. J Dairy Sci 1989;72(3):784–804. [PubMed: 2654228]
9. Halhali A, Acker GM, Grabedian M. 1,25-dihydroxyvitamin D_3 induces in vivo the decidualization of rat endometrial cells. J Reprod Fertil 1991;91(1):59–64. [PubMed: 1995862]
10. Harkness LS, Bonny AE. Calcium and vitamin D status in the adolescent: Key roles for bone, body weight, glucose tolerance, and estrogen biosynthesis. J Pediatr Adolesc Gynecol 2005;18:305–311. [PubMed: 16202933]
11. Kwiecinski GG, Petrie GI, DeLuca HF. Vitamin D is necessary for reproductive functions of the male rat. J Nutr 1989;119(5):741–744. [PubMed: 2723823]
12. Hickie JP, Lavigne DM, Woodward WD. Reduced fecundity of vitamin deficient rats. Comp Biochem Physiol 1983;74(4):923–925.
13. Kwiecinksi GG, Petrie GI, DeLuca HF. 1,25-Dihydroxyvitamin D_3 restores fertility of vitamin D-deficient female rats. Am J Physiol 1989;256(4 Pt 1):E483–E487. [PubMed: 2705521]
14. Johnson LE, DeLuca HF. Reproductive defects are corrected in vitamin D-deficient female rats fed a high calcium, phosphorus and lactose diet. J Nutr 2002;132(8):2270–2273. [PubMed: 12163674]
15. Uhland AM, Kwiecinski GG, DeLuca HF. Normalization of serum calcium restores fertility in vitamin D-deficient male rats. J Nutr 1992;122(6):1338–1344. [PubMed: 1588451]

16. Stephanou A, Ross R, Handwerger S. Regulation of human placental lactogen expression by 1,25-dihydroxyvitamin D_3. Endocrinol 1994;135(6):2651–2656.

17. Barrera D, Avila E, Hernández G, Halhali A, Biruete B, Larrea F, et al. Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol. J Steroid Biochem Mol Biol 2007;103(3–5): 529–532. [PubMed: 17257826]

18. Daftary GS, Taylor HS. Endocrine regulation of HOX genes. Endocr Rev 2006 Jun;27(4):331–355. Epub 2006 Apr 21. [PubMed: 16632680]

19. Tuan RS, Moore CJ, Brittingham JW, Kirwin JJ, Akins RE, Wong M. In vitro study of placental trophoblast calcium uptake using JEG-3 human choriocarcinoma cells. J Cell Sci 1998;98(3):333–342. [PubMed: 1829088]

20. Kurth T, Walker AM, Glynn RJ, Chan KA, Gaziano JM, Berger K, et al. Results of multivariable logistic regression, propensity matching, propensity adjustment and propensity based weighting under conditions of non union effect. Am J Epidemiol 2006;163:262–270. [PubMed: 16371515]

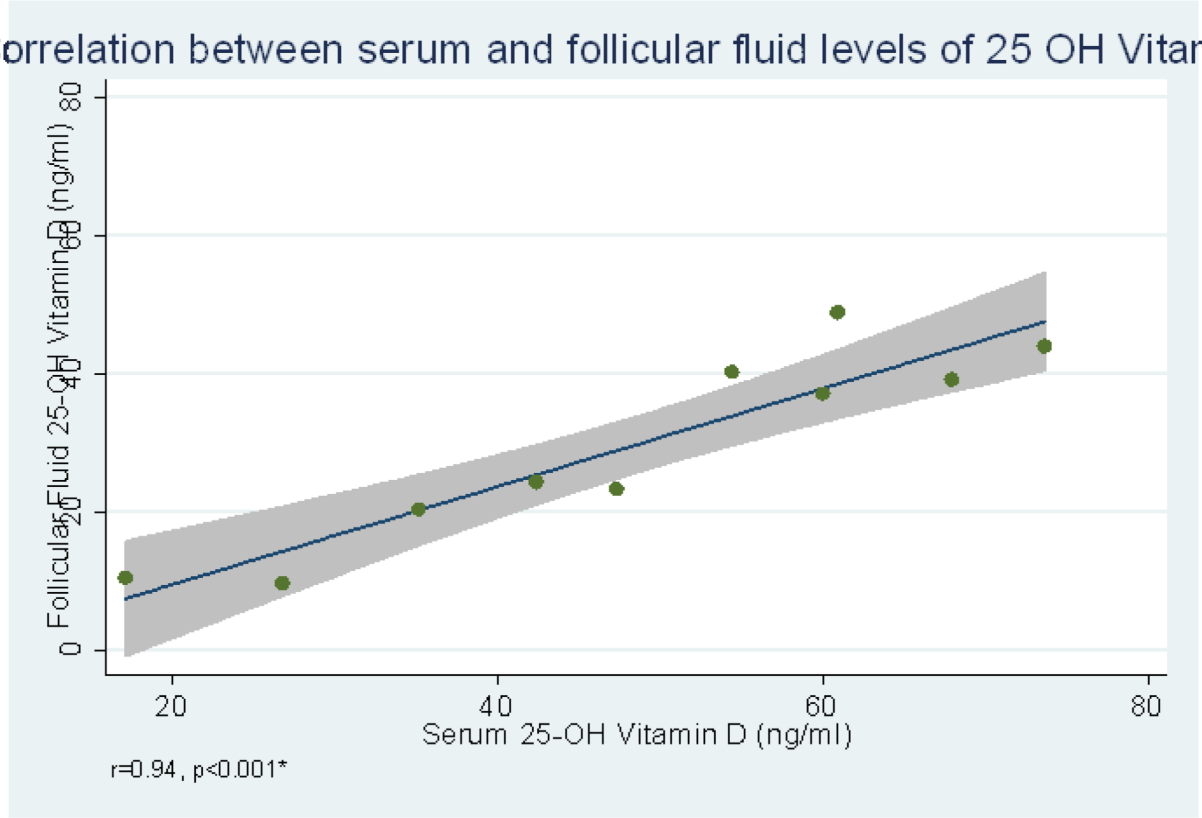


Figure 1.
Follicular fluid 25-OH Vitamin D reliably reflects serum levels

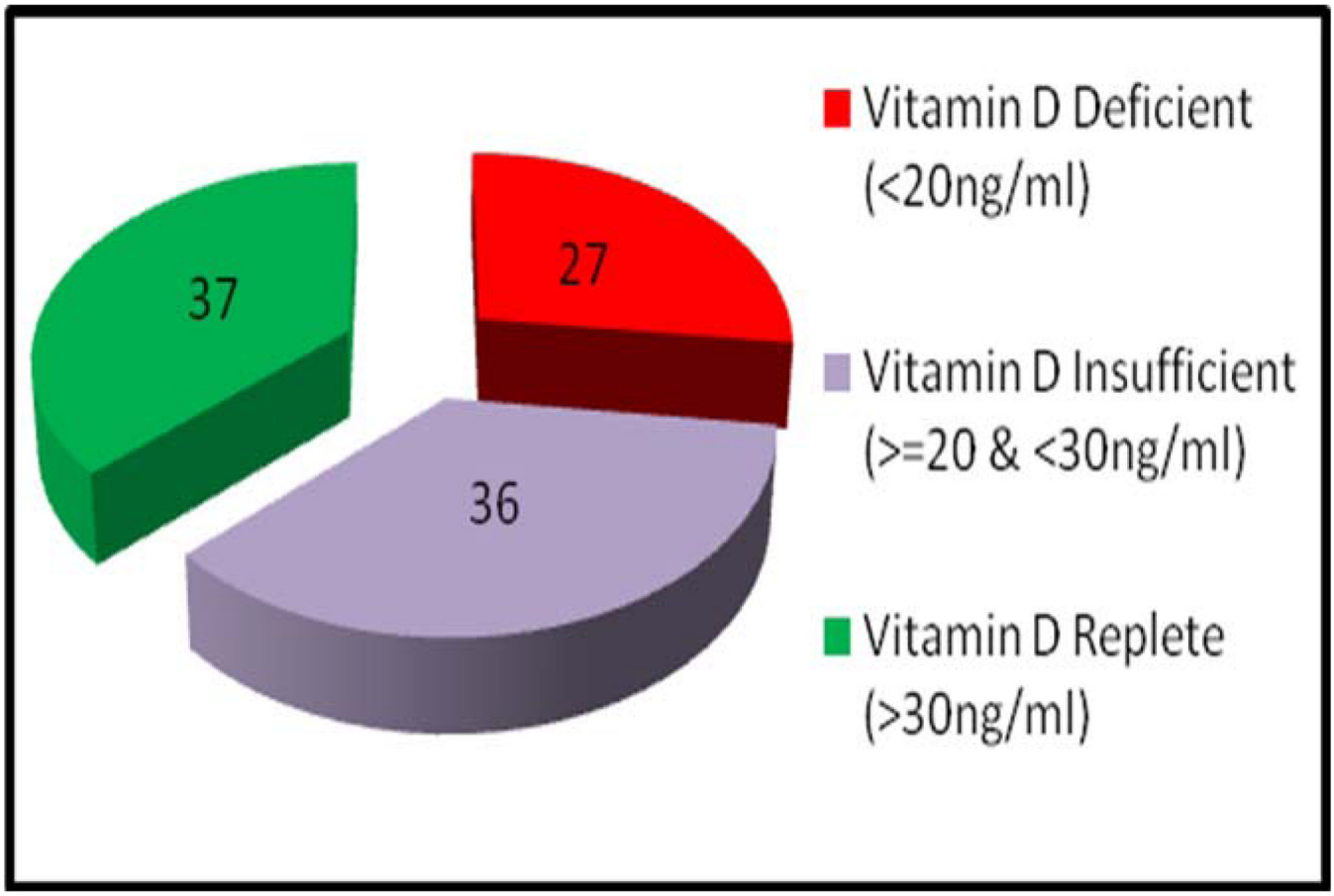


Figure 2.
Population prevalence (%) of Vitamin D status

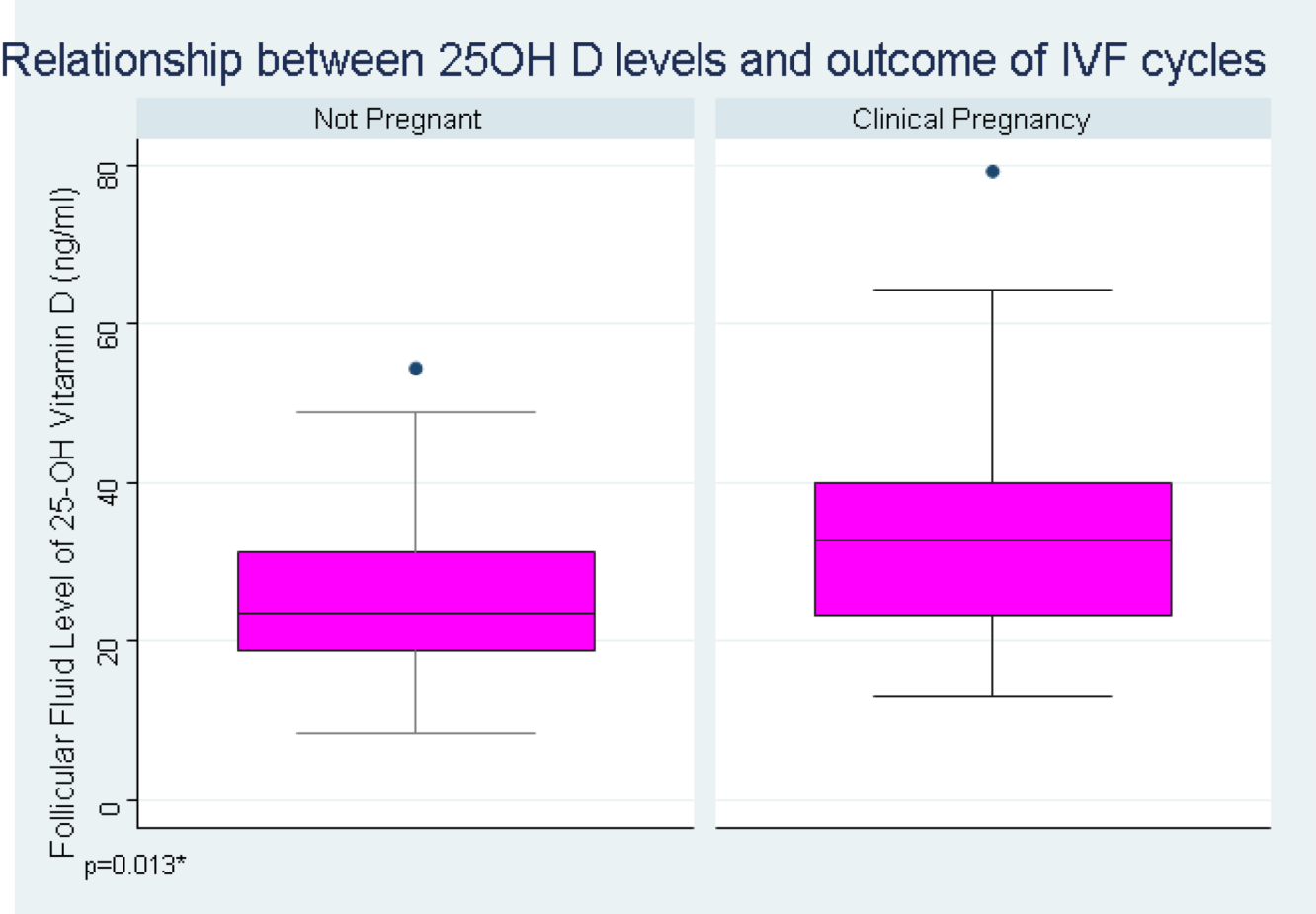
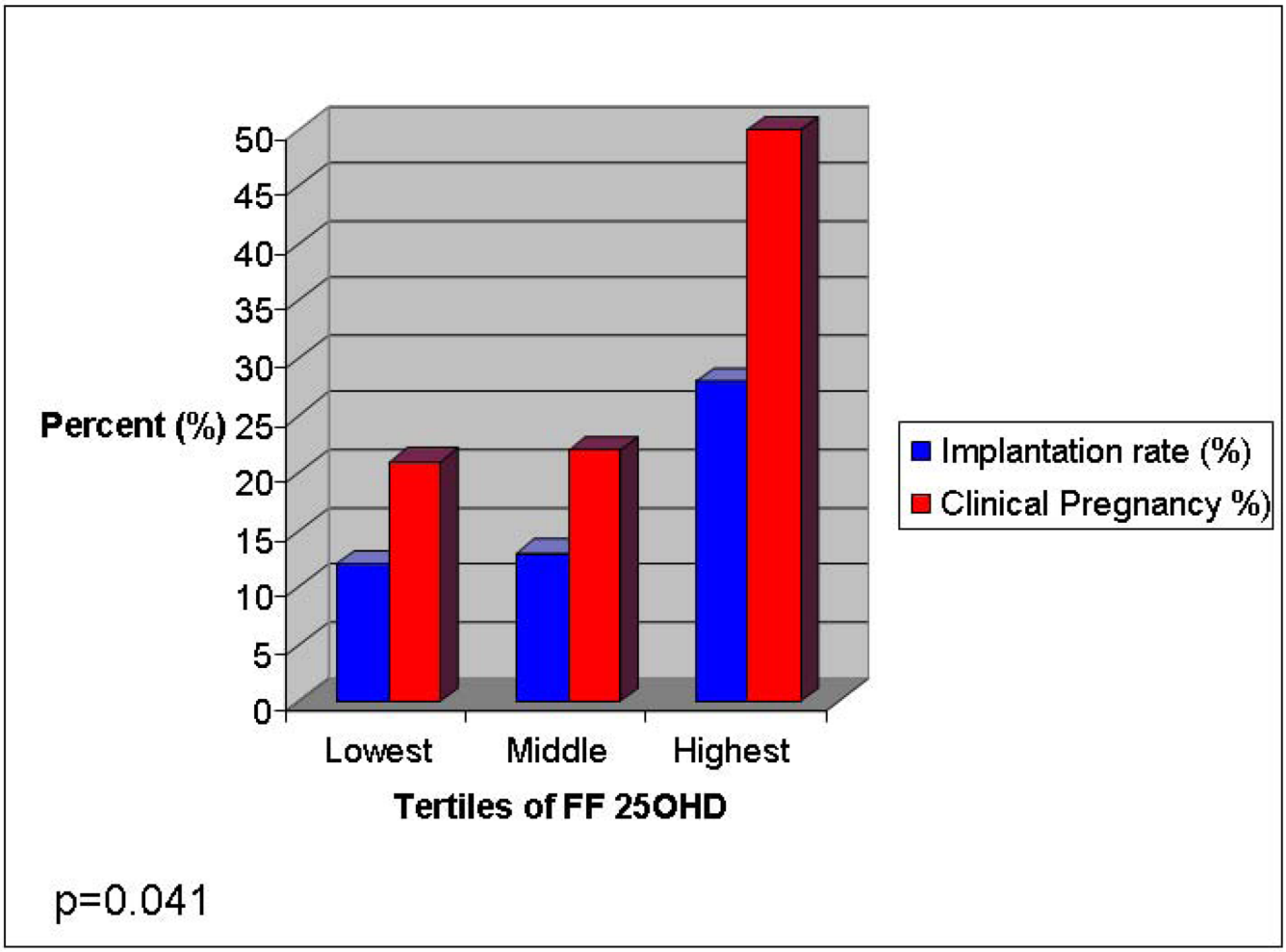


Figure 3.
Significantly higher follicular fluid 25OH Vitamin D levels are noted in IVF cycles achieving clinical pregnancy



FF 25OH D levels (ng/ml) by tertiles (mean ± SD): Lowest:16.74 ± 3.38; Middle: 25.58 ±3.17, Highest 43.01±10.65

Figure 4.
Increasing implantation and clinical pregnancy rates are observed across FF 25OHD tertiles.

Table 1

Participant and IVF cycle characteristics by outcome of IVF cycle

	Clinical Pregnancy (n=26) (30.95%)	Not Pregnant (n=58) (69.04%)	p value
FF 25OH-Vitamin D			
ng/ml	34.42 ±15.58	25.62 ± 10.53	0.013*
mmol/L	86.05 ± 38.96	64.04 ± 26.32	
Age (years)	33.88 ± 4.57	34.86± 5.08	0.565
BMI (Kg/m^2)	23.77 ± 4.00	26.37± 6.60	0.164
Race[a]			0.801
Black (%)	4/23 (17)	8/53 (15)	
White (%)	17/23 (74)	33/53 (62)	
Other Race (%)	2/23 (9)	12/53 (23)	
Baseline FSH (mIU/ml)	7.76 ±3.08	8.16 ±2.09	0.360
Gonadotropin Dose (Amps[b])	28.10 ±14.27	44.96 ±27.19	0.001*
Days of COH	10.73 ±1.43	11.93 ± 1.70	0.002*
E_2 Day of hCG (pg/ml)	2297 ± 1171.86	2266.31±1101.07	0.961
Oocytes Retrieved (n)	12.88 ± 6.33	12.02 ± 6.72	0.525
Overall Fertilization Rate (%)	53.00 ± 21.00	45.00 ± 24.00	0.154
Embryos Transferred (n)	2.56 ± 0.66	1.98 ±1.16	0.011*

Continuous data are presented as mean ± standard deviation

[a] Information on race was not available for the entire cohort.

[b] Gonadotrophin dose per ampoule= 75IU

* Statistically significant, $p<0.05$

Table 2

Predictors of successful clinical pregnancy following IVF (associations presented as odds ratio ± 95% Confidence intervals)

Clinical Pregnancy	Unadjusted OR (95% CI)	P	Adjusted OR[b] (95% CI)	P
Age (years)	0.96 (0.87–1.06)	0.404	1.01 (0.84–1.20)	0.940
Race [a]	1.71 (0.58–5.57)	0.328	1.47 (0.30–7.30)	0.635
BMI (Kg/m^2)	0.91 (0.81–1.02)	0.101	0.91 (0.78–1.06)	0.208
Embryos transferred (n)	1.75 (1.04–2.95)	0.034*	2.13(1.12–4.05)	0.021*
FF 25OH-D(ng/ml)	1.06 (1.01–1.10)	0.007*	1.07 (1.01–1.13)	0.013*

[a] White versus other races

[b] Analyses adjusted for age, BMI, race, number of embryos transferred and FF 25OH Vitamin D level

* Statistically significant, $p<0.05$