Vitamin D level is associated with increased clinical pregnancy rate and live birth rate in Chinese women undergoing IVF/ICSI-ET: a retrospective cohort study

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Abstract

Research question

To explore the relationship between serum 25(OH)D level and Clinical Pregnancy Rate (CPR) as well as Live Birth Rate (LBR) in Chinese women receiving IVF/ICSI-ET.

Design

The data of 612 patients who underwent IVF/ICSI-ET in the Reproduction Center of the Second Affiliated Hospital of Wenzhou Medical University were retrospectively analyzed.

Results

None of the baseline characteristics of participants were significantly different between the two cohorts. The trend of 25(OH)D concentration was positively correlated with CPR and LBR. Moreover, our results indicated that the younger (Age: p<0.001 both in CPR and LBR) women with primary infertility (Infertility type: p=0.004 in LBR) were more likely to get a better pregnancy outcome under the same 25(OH)D concentration stages. As shown on heatmap plots, CPR and LBR were significantly increased for 25(OH)D concentrations above 30.00ng/ml and women younger than 30 years old. The adjusted binary logistic regression and RCS showed that there exists a nonlinear positive correlation between 25(OH)D concentration and pregnancy outcome (CPR and LBR) (*nonlinear* < 0.001, respectively). The women with a sufficient 25(OH)D concentration (30ng/ml) had 1.07 (clinical pregnancy) and 1.05 (live birth) times higher successful birth outcomes compared to women with an insufficient 25(OH)D concentration (25ng/ml). ($OR_{25ng/ml,ref=30ng/ml}$ [95%CI] = 0.935[0.932-0.938] and 0.947[0.945-0.950],P<0.001, respectively)

Conclusion

In Chinese women receiving IVF/ICSI-ET, the serum level of 25(OH)D demonstrated a non-linear positive correlation with pregnancy outcome：CPR&LBR, with stronger correlations above 25 ng/ml and worse yields below 30 ng/ml. However, it could not yet be considered different in distinct ages.

Introduction

Vitamin D is mainly synthesized through the UV-B synthesis reaction of skin exposed to sunshine[1,2]. 25(OH)D deficiency is very common worldwide[3,4], and it is more obvious in Asians. According to the Chinese National Nutrition Surveillance (CNHS) [5], 25(OH)D deficiency is more frequent in women at the age of childbearing in China. This is probably attributed to sunlight time, intensity, and VDR gene polymorphisms, such as BsmI (Rs1544410) [6,7].

Researchers have so far focused on the effect of 25(OH)D on bones. However, 25(OH)D is involved in numerous mechanisms in the field of reproductive medicine. In neonates, it promotes the development of bone, nervous system, and lung[8]. In pregnant women, it attributes to endometrial calcium homeostasis maintenance, immune regulation, it has an anti-inflammatory effect during pregnancy and it reduces the risk of rickets after birth[9–11]. Moreover, research by Parikh et al. showed that 25(OH)D promotes the production of numerous hormones including estrogen, progesterone, estradiol, and insulin-like growth factor binding protein 1 (IGF-BP-1) [12,13]. Furthermore, Hahn et al. found that the 25(OH)D deficiency may be associated with insulin resistance, hyperandrogenemia polycystic ovary syndrome, and ovulation disorders[13–16].

Although the importance of 25(OH)D on fetal development and pregnancy is gradually being recognized, the effect of 25(OH)D deficiency on assisted reproductive outcomes remains unclear. The In Vitro Fertilization (IVF)/Intracytoplasmic Sperm Injection (ICSI)-EmbryoTransfer (ET) (IVF/ICSI-ET) is a traditional and common technique for assisting reproduction, but the existing studies on IVF/ICSI-ET have mainly focused on the non-Asian population. There is a lack of systematic studies on the pregnancy outcomes of Asians. Our study is thus studying the associations of the serum 25(OH)D levels in Chinese women who had received IVF/ICSI-ET with their clinical pregnancy outcomes.

Method

Patients

The patients who underwent IVF/ICSI-ET in the Second Affiliated Hospital of Wenzhou Medical University from May 2018 to December 2019 were retrospectively analyzed. This study had been ratified by the institutional review committee of the Second Affiliated Hospital of Wenzhou Medical University. All patients were consulted extensively before obtaining standard informed consent and were divided into two cohorts according to their 25(OH)D concentrations (< 30ng/ml (560), ≥ 30ng/ml(52)) and four groups for further studies (G1:<20ng/ml(284), G2:20-25ng/ml(170), G3:25-30ng/ml(106), G4:≥30ng/ml(52)).

Serum 25(OH)D measurement

All patients underwent routine serum 25(OH)D concentration measurements by liquid chromatography-mass spectrometry (LC-MS) before IVF/ICSI-ET.

IVF/ICSI-ET procedure

The long-acting protocol for ovarian stimulation was as follows: A total of 3.75 mg of gonadotropin-releasing hormone agonist (GnRH-a) was administered starting from Days 1–3 of the menstrual cycle. Inclusion criteria were examined after 35–38 days, examining whether pituitary down-regulation was achieved with E_2 < 25 pg/ml, luteinizing hormone (LH) < 5 U/L, the diameter of the ovarian follicles < 5mm, and an endometrial thickness < 5 mm. Patients who met these inclusion criteria commenced ovarian stimulation with gonadotropin of 100–300 IU daily until the day of hCG injection. The starting dose of gonadotropin was determined according to the body mass index (BMI), ovarian antral follicle count (AFC), ovarian reserve, and ovarian response in the past IVF/ICSI cycles. The dose could be adjusted for hormonal level changes and the size of the oocytes. Triggering of final follicular maturation was performed with 250μg recombinant hCG as a trigger when at least one follicle had a diameter of 18 mm or more or at least two follicles had a diameter of 17mm or more. After 35–38 hours of administration, follicles were aspirated under the guidance of transvaginal ultrasound. Embryo transfer was conducted on day five after oocyte retrieval. Clinical pregnancy was determined by visualizing a gestational sac on ultrasound at 6 weeks of gestation. Live birth was defined as having at least one alive baby born after 28 weeks of gestation.

Statistical analysis

R software (version 4.12), SPSS (version 25.0), Stata (version 15.0), GraphPad Prism (version 9.0) and MATLAB (version 2020a) were used for statistical analyses. Categorical variables were expressed as frequencies with percentages and continuous variables were shown using the medians [IQR]. Chi-square test and Fisher test were used to compare the categorical data. Mann-Whitney U test was used to compare continuous variables without normal distribution. A Scatterplot was used to study and visualize the associations between the 25(OH)D concentration, clinical pregnancy rate and birth rate. Collinearity diagnostics of variables were used to ensure the feasibility of binary logistic regression. Heatmaps were applied to visualize the associations of the combination of age and 25(OH)D concentration with CPR and LBR. The interaction analysis of binary logistic regression was used to demonstrate the absence of interactions between two variables. A binary logistic regression model was used to further explore the association between the 25(OH)D concentration, CPR and LBR adjusting other independent variables. Restricted cubic spline (RCS) was used to study the nonlinear relationship between the 25 (OH) D concentration and pregnancy outcomes. All statistical tests were bilateral, and statistically significant differences were inferred when *P-values* were less than 0.05.

Result

The 612 patients were divided into two cohorts according to their serum 25(OH)D concentrations: ≤30.0 ng/ml (insufficient group) and > 30.0 ng/ml (sufficient group). The 91.53% of women (560) were in the deficient group, while 8.50% of women (52) were in the sufficient one. There was no significant difference in the distribution of age, BMI, infertility type, duration of infertility, and AFC in the two groups ($P > 0.05$ respectively), Table 1.

Table 1
Baseline characteristics of participants in deficient and sufficient cohorts

Variable	**Total (%)**	**Deficient (%) [IQR]**	**Sufficient (%) [IQR]**	***P***
Total	612	560	52	
Age (Year)				0.276[a]
20.00–30.00	261(42.65)	27.2(43.39)[3.3]	27.8(34.62)[3.67]	
30.00–35.00	229(37.42)	32.1(37.14)[2.5]	31.8(40.38)[2.0]	
≥ 35.00	122(19.93)	36.9(19.46)[2.9]	38.5(25.0)[1.9]	
BMI (kg/m^2)				0.581[a]
<18.50	66(10.78)	17.78(10.89)[0.84]	18.05(9.62)[0.17]	
18.50–25.00	436(71.24)	21.48(71.43)[2.82]	21.16(69.23)[3.28]	
25.00–30.00	98(16.01)	26.49(15.54)[1.85]	27.1(21.15)[1.61]	
≥ 30.00	12(1.96)	31.64(2.14)[0.9]	None(0.0)[None]	
Gravid (%)				0.491[b]
Primiparity	221(36.11)	205(36.61)	16(30.77)	
Multiparity	391(63.89)	355(63.39)	36(69.23)	
Duration of infertility (Year)				0.709[a]
1–4	453(74.02)	2.0(73.93)[2.0]	2.0(75.0)[2.0]	
5–8	127(20.75)	6.0(21.07)[1.0]	7.0(17.31)[3.0]	
9–12	28(4.58)	10.0(4.46)[1.0]	10.0(5.77)[1.5]	
13–16	4(0.65)	13.0(0.54)[0.5]	16.0(1.92)[0.0]	
AFC (number)				0.805[a]

AFC, antral follicle counting; BMI: body mass index; IQR = interquartile range; CPR: Clinical pregnancy rate

Unless otherwise stated, values are presented as median (interquartile)

[a] Mann-Whitney *U* test.

[b] Pearson χ^2

*Indicates a significant (P < 0.05) difference between deficient and sufficient cohorts

**Indicates an extremely significant (P < 0. 001) difference between deficient and sufficient cohorts

Variable	Total (%)	Deficient (%) [IQR]	Sufficient (%) [IQR]	*P*
□9	56(9.15)	6.0(8.75)[2.0]	5.0(13.46)[1.5]	
9–19	350(57.19)	14.0(57.5)[5.0]	13.5(53.85)[5.0]	
□19	206(33.66)	24.0(33.75)[6.0]	25.0(32.69)[5.0]	

AFC, antral follicle counting; BMI: body mass index; IQR = interquartile range; CPR: Clinical pregnancy rate

Unless otherwise stated, values are presented as median (interquartile)

[a] Mann-Whitney *U* test.

[b] Pearson χ^2

*Indicates a significant (P < 0.05) difference between deficient and sufficient cohorts

**Indicates an extremely significant (P < 0. 001) difference between deficient and sufficient cohorts

The two groups were further divided into four subgroups using the following thresholds: □20.0 ng/ml (G1), 20.0–25.0 ng/ml (G2), 25.0–30.0 ng/ml (G3)], > 30.0 ng/ml (G4)]. Table 2 and Table 3 are presenting the baseline characteristics in pregnancy outcomes. The distribution of age was significantly different in pregnancy outcomes ($P < 0.001$ CPR and LBR) and in the infertility type in LBR ($P < 0.004$). Women of younger age or primary infertility may present better pregnancy outcomes compared to women with acquired infertility.

Table 2
Characteristics of cohorts' outcome about CPR

Variable	Total (%)	CPS (%) [IQR]	CPF (%) [IQR]	*P*	CPR
Total	612	371	241		60.62
25(OH)D C(ng/ml)				0.592^{a}	
0–20.00	284(46.40)	15.64(45.01) [3.46]	16.58(48.55) [3.76]		58.80
20.00–25.00	170(27.78)	22.30(27.49) [2.66]	22.12(28.22) [2.31]		60.00
25.00–30.00	106(17.32)	26.88(16.98) [2.08]	26.78(17.84) [2.14]		59.43
≥ 30.00	52(8.50)	32.54(10.51) [3.07]	35.08(5.39) [2.91]		75.00
Age (Year)				$< 0.001^{a**}$	
20.00–30.00	261(42.65)	27.1(47.44) [3.40]	27.5(35.27)[3.0]		67.43
30.00–35.00	229(37.42)	31.9(37.74)[2.3]	32.1(36.93)[2.5]		61.14
≥ 35.00	122(19.93)	37.3(14.82)[3.4]	36.9(27.8)[2.6]		45.08
BMI (kg/m^2)				0.514^{a}	
<18.50	66(10.78)	17.87(9.7)[0.48]	17.71(12.45)[0.86]		54.55
18.50–25.00	436(71.24)	21.41(73.85)[2.86]	21.48(67.22)[2.73]		62.84
25.00–30.00	98(16.01)	26.65(15.09) [1.67]	26.55(17.43) [1.79]		57.14
≥ 30.00	12(1.96)	31.18(1.35) [1.03]	31.64(2.9) [0.57]		41.67
Infertility type (%)				0.058^{b}	
PI	221(36.11)	145(39.08)	76(31.54)		65.61
AI	391(63.89)	226(60.91)	165(68.46)		57.80
Duration of infertility (Year)				0.258^{a}	

CPS, Clinical pregnancy success; CPF, Clinical pregnancy failure; 25(OH)D C: 25(OH)D concentration; AFC, antral follicle counting; BMI: body mass index; PI, Primary infertility; AI, acquired infertility; IQR = interquartile range; CPR: Clinical pregnancy rate

Unless otherwise stated, values are presented as median (interquartile)

[a] Mann-Whitney *U* test.

[b] Pearson χ^2

*Indicates a significant (P < 0.05) difference between CPS and CPF

**Indicates an extremely significant (P < 0. 001) difference between CPS and CPF

Variable	Total (%)	CPS (%) [IQR]	CPF (%) [IQR]	*P*	CPR
1–4	453(74.02)	2.0(77.36) [2.00]	2.0(68.88) [2.00]		63.58
5–8	127(20.75)	6.0(18.33) [2.00]	6.0(24.48) [1.00]		53.54
9–12	28(4.58)	10.0(3.77) [1.00]	10.0(5.81) [0.75]		50.00
13–16	4(0.65)	14.5(0.54) [1.50]	13.5(0.83) [0.50]		50.00
AFC (number)				0.045^{a*}	
□9	56.0(9.15)	6.5(9.16) [1.00]	6.0(9.13) [2.50]		60.71
9–19	350(57.19)	14.0(54.72) [5.00]	14.0(61.00) [5.50]		58.00
□19	206(33.66)	24.5(36.12) [6.75]	23.0(29.88) [4.00]		65.05

CPS, Clinical pregnancy success; CPF, Clinical pregnancy failure; 25(OH)D C: 25(OH)D concentration; AFC, antral follicle counting; BMI: body mass index; PI, Primary infertility; AI, acquired infertility; IQR = interquartile range; CPR: Clinical pregnancy rate

Unless otherwise stated, values are presented as median (interquartile)

^{a} Mann-Whitney *U* test.

^{b} Pearson χ^2

*Indicates a significant (P < 0.05) difference between CPS and CPF

**Indicates an extremely significant (P < 0. 001) difference between CPS and CPF

Table 3
Characteristics of cohorts’ outcome about BR

Variable	Total (%)	LB (%) [IQR]	ABN (%) [IQR]	*P*	BR
Total	612	341	271		55.72
25(OH)D C(ng/ml)				0.578[a]	
0–20.00	248(46.41)	15.63(44.87)[3.47]	16.52(48.34)[3.6]		53.87
20.00–25.00	170(27.78)	22.41(27.86)[2.7]	2.15(27.68)[2.2]		55.88
25.00–30.00	106(17.32)	26.96(17.01)[2.23]	26.58(17.71)[1.93]		54.72
≥ 30.00	52(8.5)	32.54(10.26)[3.47]	34.57(6.27)[3.06]		67.31
Age (Year)				< 0.001[a**]	
20.00–30.00	261(42.65)	27.0(49.56)[3.4]	27.55(33.95)[2.95]		64.75
30.00–35.00	229(37.42)	31.9(37.54)[2.45]	32.1(37.27)[2.3]		55.9
≥ 35.00	122(19.93)	37.45(12.9)[3.4]	36.8(28.78)[2.5]		36.07
BMI (kg/m^2)				0.237[a]	
<18.50	66(10.78)	17.83(9.97)[0.57]	17.71(11.81)[0.85]		51.52
18.50–25.00	436(71.24)	21.48(74.78)[2.85]	21.45(66.79)[2.7]		58.49
25.00–30.00	98(16.01)	26.64(14.37)[1.71]	26.62(18.08)[1.88]		50.0
≥ 30.00	12(1.96)	31.18(0.88)[0.53]	31.64(3.32)[0.78]		25.0
Infertility type (%)				0.004[b*]	
PI	221(36.11)	140(41.06)	81(29.89)		63.35
AI	391(63.89)	201(58.94)	190(70.11)		51.41
Duration of infertility (Year)				0.097[a]	

LB, Live birth; ABN, abortion; 25(OH)D C: 25(OH)D concentration; AFC, antral follicle counting; PI, Primary infertility; AI, acquired infertility; BMI: body mass index; IQR = interquartile range;

Unless otherwise stated, values are presented as median (interquartile)

[a] Mann-Whitney *U* test.

[b] Pearson χ^2

*Indicates a significant (P < 0.05) difference between LB and ABN

**Indicates an extremely significant (P < 0. 001) difference between LB and ABN

Variable	Total (%)	LB (%) [IQR]	ABN (%) [IQR]	*P*	BR
1–4	453(74.02)	2.0(78.01)[2.0]	2.0(69.0)[2.0]		58.72
5–8	127(20.75)	6.0(18.18)[2.0]	6.0(23.99)[1.0]		48.82
9–12	28(4.58)	10.0(3.23)[1.0]	10.0(6.27)[1.0]		39.29
13–16	4(0.65)	14.5(0.59)[1.5]	13.5(0.74)[0.5]		50.0
AFC (number)				0.066[a]	
9	56(9.15)	7.0(8.8)[1.75]	6.0(9.59)[2.0]		53.57
9–19	350(57.19)	14.0(55.13)[5.0]	14.0(59.78)[5.75]		53.71
19	206(33.66)	25.0(36.07)[6.0]	23.0(30.63)[4.5]		59.71

LB, Live birth; ABN, abortion; 25(OH)D C: 25(OH)D concentration; AFC, antral follicle counting; PI, Primary infertility; AI, acquired infertility; BMI: body mass index; IQR = interquartile range;

Unless otherwise stated, values are presented as median (interquartile)

[a] Mann-Whitney *U* test.

[b] Pearson χ^2

*Indicates a significant (P < 0.05) difference between LB and ABN

**Indicates an extremely significant (P < 0. 001) difference between LB and ABN

Figure1. shown that CPR and LBR in G4 were significantly higher than those in G1-G3, suggesting that there might be a nonlinear relationship between the 25(OH)D concentration and both CPR and LBR.

As shown in Table 4, there was no multicollinearity among the respective variables (all VIF < 5) according to multivariable analysis.

Table 4
Multicollinearity test results of all variables used for logistic regression

Variable	Tolerance	VIF
25(OH)D C(ng/ml)	0.973	1.028
Age (Year)	0.850	1.176
BMI (kg/m^2)	0.970	1.031
Infertility type (%)	0.913	1.096
Duration of infertility (Year)	0.940	1.063
AFC (number)	0.943	1.061
CPR: Clinical pregnancy rate		

Heatmaps were provided to visualize the relationship between age, 25(OH)D concentration and CPR (Fig. 2A) and LBR (Fig. 2B). As shown in Fig. 2, younger participants or ones with higher 25(OH)D concentrations presented higher CPR or LBR. However, when binary logistic regression models were used and considering interaction terms to explore the interaction between the variables with significant differences in baseline characteristics (Table 1.) and 25(OH)D concentration, the results showed that neither age nor gravid had an interaction with 25(OH)D concentration. ($P > 0.05$ in Fig. 3.).

The results of the association between 25(OH)D concentration and CPR in our study are shown in Table 5. In the unadjusted model, P for Trend = 0.112, taking G4 as the reference, $P < 0.05$ for G1, and its Odds Ratios (95% CI) was 0.476 (0.243–0.930)., The results of binary logistic regression analysis showed statistically significant differences for the model adjusted for Age and AFC (Model1) and the model adjusted for all covariates (Model2). *P-values* for the trendlines in Model 1 and Model 2 were 0.038 and 0.043, respectively. The Odds Ratios (95% CI) of G1, G2 and G3 in Model1 against G4 were 0.417 (0.220–0.835), 0.472 (0.233–0.960) and 0.469 (0.223–0.990), respectively. In model 2, the Odds Ratios (95% CI) of G1, G2 and G3 against G4 were 0.414 (0.208–0.824), 0.457 (0.224–0.932) and 0.457 (0.215–0.971), respectively.

Table 5
Logistic regression analyses of the relationship between concentration of 25(OH)D and CPR

Variable	Crude		Model I[a]		Model II[b]	
	OR (95%CI)	*P*-Value	OR (95%CI)	*P*-Value	OR (95%CI)	*P*-Value
Groups[&]						
G1	0.476(0.243–0.930)	0.030	0.417(0.220–0.835)	0.012	0.414(0.208–0.824)	0.012
G2	0.500(0.249–1.006)	0.052	0.472(0.233–0.960)	0.038	0.457(0.224–0.932)	0.031
G3	0.488(0.234–1.021)	0.057	0.469(0.223–0.990)	0.047	0.457(0.215–0.971)	0.042
G4	1.000		1.000		1.000	
P for trend		0.112		0.038		0.043
The last group is used as the respective reference group						
a: Adjust for age and AFC						
b: Adjust for age, BMI, AFC, gestation and duration of infertility						
OR, odds ratio; CI, confidence interval;						
&: All groups are based on Table 1.						

Table 6. shows the results of exploring the relationship between 25(OH)D concentration and Birth Rate in the participating population. In the unadjusted model, the relationship between each group and Birth Rate was not found to be statistically significant, with the p-value of the trendline being 0.179. The p-value of the trendline of the binary logistic regression analysis for adjusted for Age, AFC and gravid model 1 was 0.036. G4 as the reference, A statistically significant difference was found between G1 and G4, which was used as reference, regarding their association with LBR, with the odds ratio (95% CI) being 0.469 (0.246–0.895). The *p-value* of the trendline of Model 2, which was adjusted for all covariates, was 0.052. A statistically significant difference was found between G1 and G4, which was used as reference, regarding their association with LBR, with the odds ratio (95% CI) being0.472 (0.246–0.904).

Table 6

Logistic regression analyses of the relationship between concentration of 25(OH)D and BR

Variable	**Crude**		**Model I[a]**		**Model II[b]**	
	OR (95%CI)	*P*-Value	OR (95%CI)	*P*-Value	OR (95%CI)	*P*-Value
Groups[&]						
G1	0.567(0.304–1.059)	0.075	0.469(0.246–0.895)	0.022	0.472(0.246–0.904)	0.023
G2	0.615(0.320–1.183)	0.145	0.565(0.288–1.106)	0.096	0.552(0.281–1.086)	0.085
G3	0.587(0.293–1.175)	0.132	0.567(0.278–1.155)	0.118	0.540(0.264–1.106)	0.092
G4	1.000		1.000		1.000	
P for trend		0.179		0.036		0.052

The last group is used as the respective reference group

a: Adjust for age, AFC, infertility type

b: Adjust for age, BMI, AFC, gestation and duration of infertility

OR, odds ratio; CI, confidence interval;

&: All groups are based on Table 2.

Figure 4. depicts the dose-response curve when the predicting the Odds Ratio value of clinical pregnancy and live birth using the binary logistic regression model. Model 2 was used as the dependent variable and 25(OH)D concentration (continuous variable) of the participating population was used as the independent variable. The fitting method used was the restricted cubic spline curve (RCS). The dose-effect relationship curve suggested that there was a nonlinear positive correlation between the 25(OH)D concentration and pregnancy outcome (P for nonlinear < 0.001, respectively). CPR and LBR increased with the increase of 25(OH)D concentration. For example, taking sufficient 25(OH)D concentration (30ng/ml) as reference, the women with a sufficient 25(OH)D concentration (30ng/ml) presented 1.07 times increased clinical pregnancy rates and 1.05 times increased live birth rates compared to women with an insufficient 25(OH)D concentration (25ng/ml). ($OR_{25ng/ml,}$) [95%CI] = 0.935[0.932–0.938] and 0.947[0.945–0.950], P < 0.001, respectively). In the range of > 30 ng/ml, the positive correlation effect of 25(OH)D concentration on CPR and LBR was stronger than the one below 30 ng/ml. However, when the serum 25(OH)D concentration was lower than 25ng/ml, the OR value remained relatively stable. ($OR_{20ng/ml}$) [95%CI] = 0.931[0.926–0.936] and 0.941[0.936–0.946], P < 0.001, respectively), ($OR_{15ng/ml,}$) [95%CI] = 0.913[0.909–0.918] and 0.913[0.909–0.917], P < 0.001, respectively).

Discussion

Infertility is a disease and social problem. According to the reports[9], about 10–12% of couples are suffering from infertility worldwide. In China, relevant studies have shown[9–11] that the prevalence of infertility greatly varies among different regions, with the prevalence among women of childbearing age ranging from 6.7–25%. One of the current methods to solve this problem is to conduct IVF for women of childbearing age with confirmed infertility. Therefore, researchers have been exploring how to obtain a good pregnancy outcome of IVF/ICSI-ET conveniently and effectively.

Current systematic studies suggest that Vitamin D may have some positive effects during normal pregnancy. For example, Vitamin D can promote the development of the nervous[17–19], motor[8,17,20], and respiratory[21] systems of the fetus, respectively, and regulate the immunity[22] anti-infection, and allergy. In contrast, lower serum 25(OH)D in pregnant women has been linked to a higher risk of preterm birth (PTB) or intrauterine growth retardation (IUGR) [23].

Our study set 30ng/ml as sufficient 25(OH)D concentrations, as defined by the Italian Association of Clinical Endocrinologists in 2018[24], which recommended maintaining the 25(OH)D levels above 30 ng/mL (75 nmol/L). Furthermore, Rudick B reported racial differences in the effect of 25(OH)D concentrations on clinical pregnancy rates. However, the 25(OH)D deficiency was associated with a lower pregnancy rate among non-Hispanic whites, but not among Asians, with the authors suggesting that this might be attributed to the lower success rate of IVF-ET in Asia[25]. The present study is thus the first to investigate the relationship between serum 25(OH)D levels and CPR in IVF/ICSI-ET in Chinese populations. 25(OH)D was significantly positively associated with CPR. This result has been supported by similar studies. For instance, a meta-analysis showed[26] that among all women who underwent IVF/ICSI-ET in their analysis, women with ample 25(OH)D were more likely to have a good clinical pregnancy outcome than women with deficient or insufficient 25(OH)D. Furthermore, Jing Zhao et al. reached a similar conclusion that 25(OH)D sufficiency increased live birth rates as an IVF/ICSI-ET outcome[27]. However, to the best of our knowledge, none of the existing studies have reported the nonlinear positive correlation yet.

The results of the present study were supported by many molecular mechanisms. Vitamin D promotes placental calcium transport, stimulating the expression of prolactin and decidualization, regulating the expression of HOXA 10, a target gene that has been associated with the implantation process[28–32]. Another experimental study reported that the lack of the VDR gene is associated with gonadal insufficiency, down-regulation of aromatase gene expression, low aromatase activity, hypergonadotropic hypogonadism[32,33], and features of estrogen deficiency, such as bone malformations, uterine hypoplasia, impaired folliculogenesis, and infertility[32,34].

Furthermore, our study showed that age remains an important factor in IVF/ICSI-ET. In the heat map of Fig. 2A the OR was found to exceed 10 and the color changed 30 times faster for women younger. Changes in the same 25(OH)D levels had a more positive effect on changes in pregnancy outcomes in women under 30 years of old. A similar conclusion related to LBR is shown in Fig. 2B. Therefore, we hypothesize that women below the age of 30 are more sensitive to Vitamin D deficiency.

However, our research results were still controversial. Jason M. Franasiak's study showed that the effect of 25(OH)D on pregnancy outcomes was insignificant[35]. Abbas Aflatoonian suggested that 25(OH)D deficiency treatment did not relate to a higher pregnancy rate in the cycle of frozen-thawed embryo transfer[36]. Arne van de Vijver[37] and Liu Jiang[13] also reached the similar conclusion of no significant correlation between the two. The process of IVF/ICSI-ET is complex, and many factors can affect outcomes. Combined with our findings, it is suggested that this difference could be explained from the perspective of age, since the clinical pregnancy outcomes of women over 30 years old, especially the elderly pregnant women (age ≥ 35 years old), are affected by other more important factors implying the presence of a bias in our model. For example, research by Michaël De Brucker has shown that clinical pregnancy rates in older pregnant women were driven by several complications. The incidence of pregnancy-induced hypertension was only 6–8% in the general pregnant women group, while it increased to 15% in the elderly pregnant women[38]. In addition, it is obvious that women's fertility decreases with the increase in age[39] with a notable change being the deterioration of the ovarian reserve function with age. Therefore, AFC, as one of the indicators for evaluating the ovarian reserve function[40], showed a downward trend with aging[41]. Moreover, the decline in ovarian function adversely affects the outcome of IVF/ICSI-ET. Therefore, if the patients were not grouped by age, it would have been very likely to obtain different results. Moreover, as shown in our study, the relationship between 25(OH)D and pregnancy outcomes of IVF/ICSI-ET patients is nonlinear. This means that if the patient's 25(OH)D levels were not well grouped or mainly distributed below 25ng/ml, it would be difficult to observe the correct conclusion.

In summary, our study was the first case study on the Chinese population to propose the CPR and LBR as the research outcome of IVF/ICSI-ET, that explored the trends of pregnancy outcomes under the combined effects of covariates such as age. According to PubMed, we were the first to describe this nonlinear relationship using RCS curves. This was important for the subsequent development of IVF/ICSI-ET and for patients to obtain a good pregnancy outcome. To some extent, it bridged and explained previous controversial views in this field. However, our study still possesses some limitations. First, it only included female patients administered by the Second Affiliated Hospital of Wenzhou Medical University. Second, it had been suggested[42] that seasonal variations may affect circulating 25-(OH)D levels, and we did not rule out these possible interferences in this study. In addition, there were many factors affecting the pregnancy outcome. For example, measured in early pregnancy 25(OH)D levels are likely to fail to better represent 25(OH)D levels in late pregnancy, weakening thus the link between 25(OH)D levels and LBR compared to CPR. Further research could promote understanding of the role and influence of 25-(OH)D in IVF/ICSI-ET better and finally contribute to the identification of methods to improve IVF/ICSI-ET pregnancy outcome.

Conclusion

Serum 25(OH)D level was significantly related to pregnancy outcome(CPR&LBR)in Chinese women receiving IVF/ICSI-ET with a nonlinear positive correlation. When 25(OH)D levels exceeded 25 ng/ml, the correlation appeared stronger, while insufficient 25(OH)D (under 30ng/ml) deliver a worse pregnancy outcome. However, their does not exist substantial evidence to allow considering different 25(OH)D levels in distinct ages.

Abbreviations

IVF-ET/ICSI: In Vitro Fertilization - Embryo Transfer/Intracytoplasmic Sperm Injection

IVF-ET: In Vitro Fertilization - Embryo Transfer

ICSI: Intracytoplasmic Sperm Injection

CPR: Clinical Pregnancy Rate

LBR: Live Birth Rate

RCS: Restricted cubic spline

OR: Odds ratio

BMI: Body mass index

CNHS: Chinese national nutrition surveillance

IGF-BP-1: Insulin-like growth factor binding protein 1

LC-MS: Liquid chromatography-mass spectrometry

GnRH-a: Gonadotropin-releasing hormone agonist

hCG: Human chorionic gonadotrophin

CO_2: Carbon dioxide

O_2: Oxygen

ICM: Inner cell mass

IQR: Inter-quartile range

CIs: Confidence intervals

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Contributions

Z.Y., P.W. and J.Z. contributed to the concept and design of the study. Z.Y. conceived, analyzed results, and wrote/edited articles. P.W. and Y.H. tested and analyzed the results. Y.S. collected the data. J.X. and Y.S. provided assistance with certain aspects of data analysis. Z.L. and L.X. involved in the revision of the manuscript. H.C. and Y.Z. conceived the study and participated in its design, coordination, and article modification. Z.L. contributed to the revision of the manuscript. The author read and approved the final draft .

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Ethics declarations

Ethics approval and consent to participate

This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University (LCKY2018-38).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Dataset Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Hossein-nezhad, A. & Holick, M. F. Vitamin D for health: a global perspective. Mayo Clin Proc **88**, 720–755, doi:10.1016/j.mayocp.2013.05.011 (2013).
2. Wacker, M. & Holick, M. F. Sunlight and Vitamin D: A global perspective for health. Dermatoendocrinol **5**, 51–108, doi:10.4161/derm.24494 (2013).
3. Nesby-O'Dell, S. *et al.* Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr **76**, 187–192, doi:10.1093/ajcn/76.1.187 (2002).
4. Yu, S. *et al.* The high prevalence of hypovitaminosis D in China: a multicenter vitamin D status survey. Medicine (Baltimore) **94**, e585, doi:10.1097/md.0000000000000585 (2015).
5. Hu, Y. *et al.* Vitamin D Nutritional Status of Chinese Pregnant Women, Comparing the Chinese National Nutrition Surveillance (CNHS) 2015–2017 with CNHS 2010–2012. Nutrients **13**, doi:10.3390/nu13072237 (2021).
6. Dovnik, A. & Mujezinović, F. The Association of Vitamin D Levels with Common Pregnancy Complications. Nutrients **10**, doi:10.3390/nu10070867 (2018).
7. Ginde, A. A., Sullivan, A. F., Mansbach, J. M. & Camargo, C. A., Jr. Vitamin D insufficiency in pregnant and nonpregnant women of childbearing age in the United States. Am J Obstet Gynecol **202**, 436.e431-438, doi:10.1016/j.ajog.2009.11.036 (2010).
8. Ala-Houhala, M., Koskinen, T., Terho, A., Koivula, T. & Visakorpi, J. Maternal compared with infant vitamin D supplementation. Arch Dis Child **61**, 1159–1163, doi:10.1136/adc.61.12.1159 (1986).
9. Cui, C., Wang, L. & Wang, X. Effects of Self-Esteem on the Associations Between Infertility-Related Stress and Psychological Distress Among Infertile Chinese Women: A Cross-Sectional Study. Psychol Res Behav Manag **14**, 1245–1255, doi:10.2147/prbm.S326994 (2021).

10. Zhou, Z. *et al.* Epidemiology of infertility in China: a population-based study. Bjog **125**, 432–441, doi:10.1111/1471-0528.14966 (2018).
11. Junqing, W. *et al.* Reference value of semen quality in Chinese young men. Contraception **65**, 365–368, doi:10.1016/s0010-7824(02)00281-0 (2002).
12. Parikh, G. *et al.* Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. Horm Metab Res **42**, 754–757, doi:10.1055/s-0030-1262837 (2010).
13. Jiang, L., Ji, L., Song, J. & Qian, K. The effect of serum vitamin D levels in couples on embryo development and clinical outcomes. Reprod Biomed Online **38**, 699–710, doi:10.1016/j.rbmo.2018.12.036 (2019).
14. Hahn, S. *et al.* Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes **114**, 577–583, doi:10.1055/s-2006-948308 (2006).
15. Ngo, D. T. *et al.* Determinants of insulin responsiveness in young women: Impact of polycystic ovarian syndrome, nitric oxide, and vitamin D. Nitric Oxide **25**, 326–330, doi:10.1016/j.niox.2011.06.005 (2011).
16. Wehr, E. *et al.* Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. Eur J Endocrinol **161**, 575–582, doi:10.1530/eje-09-0432 (2009).
17. Larqué, E., Morales, E., Leis, R. & Blanco-Carnero, J. E. Maternal and Foetal Health Implications of Vitamin D Status during Pregnancy. Ann Nutr Metab **72**, 179–192, doi:10.1159/000487370 (2018).
18. Eyles, D., Brown, J., Mackay-Sim, A., McGrath, J. & Feron, F. Vitamin D3 and brain development. Neuroscience **118**, 641–653, doi:10.1016/s0306-4522(03)00040-x (2003).
19. Kalueff, A. V. *et al.* The vitamin D neuroendocrine system as a target for novel neurotropic drugs. CNS Neurol Disord Drug Targets **5**, 363–371, doi:10.2174/187152706784111506 (2006).
20. Viljakainen, H. T. *et al.* Maternal vitamin D status determines bone variables in the newborn. J Clin Endocrinol Metab **95**, 1749–1757, doi:10.1210/jc.2009-1391 (2010).
21. Lykkedegn, S., Sorensen, G. L., Beck-Nielsen, S. S. & Christesen, H. T. The impact of vitamin D on fetal and neonatal lung maturation. A systematic review. Am J Physiol Lung Cell Mol Physiol **308**, L587-602, doi:10.1152/ajplung.00117.2014 (2015).
22. Liu, N. Q. & Hewison, M. Vitamin D, the placenta and pregnancy. Arch Biochem Biophys **523**, 37–47, doi:10.1016/j.abb.2011.11.018 (2012).
23. Wei, S. Q., Qi, H. P., Luo, Z. C. & Fraser, W. D. Maternal vitamin D status and adverse pregnancy outcomes: a systematic review and meta-analysis. J Matern Fetal Neonatal Med **26**, 889–899, doi:10.3109/14767058.2013.765849 (2013).
24. Cesareo, R. *et al.* Italian Association of Clinical Endocrinologists (AME) and Italian Chapter of the American Association of Clinical Endocrinologists (AACE) Position Statement: Clinical Management of Vitamin D Deficiency in Adults. Nutrients **10**, doi:10.3390/nu10050546 (2018).

25. Rudick, B. *et al.* Characterizing the influence of vitamin D levels on IVF outcomes. Hum Reprod **27**, 3321–3327, doi:10.1093/humrep/des280 (2012).

26. Chu, J. *et al.* Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis. Hum Reprod **33**, 65–80, doi:10.1093/humrep/dex326 (2018).

27. Zhao, J. *et al.* Whether vitamin D was associated with clinical outcome after IVF/ICSI: a systematic review and meta-analysis. Reprod Biol Endocrinol **16**, 13, doi:10.1186/s12958-018-0324-3 (2018).

28. Heaney, R. P. Vitamin D in health and disease. Clin J Am Soc Nephrol **3**, 1535–1541, doi:10.2215/cjn.01160308 (2008).

29. Stephanou, A., Ross, R. & Handwerger, S. Regulation of human placental lactogen expression by 1,25-dihydroxyvitamin D3. Endocrinology **135**, 2651–2656, doi:10.1210/endo.135.6.7988455 (1994).

30. Barrera, D. *et al.* Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol. J Steroid Biochem Mol Biol **103**, 529–532, doi:10.1016/j.jsbmb.2006.12.097 (2007).

31. Daftary, G. S. & Taylor, H. S. Endocrine regulation of HOX genes. Endocr Rev **27**, 331–355, doi:10.1210/er.2005-0018 (2006).

32. Ozkan, S. *et al.* Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertil Steril **94**, 1314–1319, doi:10.1016/j.fertnstert.2009.05.019 (2010).

33. Kinuta, K. *et al.* Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology **141**, 1317–1324, doi:10.1210/endo.141.4.7403 (2000).

34. Yoshizawa, T. *et al.* Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet **16**, 391–396, doi:10.1038/ng0897-391 (1997).

35. Franasiak, J. M. *et al.* Vitamin D levels do not affect IVF outcomes following the transfer of euploid blastocysts. Am J Obstet Gynecol **212**, 315.e311-316, doi:10.1016/j.ajog.2014.09.029 (2015).

36. Aflatoonian, A., Arabjahvani, F., Eftekhar, M. & Sayadi, M. Correction to "Effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles: A randomized clinical trial" [Iran J Reprod Med 2014; 12: 595–600]. *Int J Reprod Biomed* **20**, 68, doi:10.18502/ijrm.v20i1.10410 (2022).

37. van de Vijver, A. *et al.* Vitamin D deficiency and pregnancy rates following frozen-thawed embryo transfer: a prospective cohort study. Hum Reprod **31**, 1749–1754, doi:10.1093/humrep/dew107 (2016).

38. De Brucker, M. *et al.* Assisted reproduction counseling in women aged 40 and above: a cohort study. J Assist Reprod Genet **30**, 1431–1438, doi:10.1007/s10815-013-0085-z (2013).

39. Broekmans, F. J., Soules, M. R. & Fauser, B. C. Ovarian aging: mechanisms and clinical consequences. Endocr Rev **30**, 465–493, doi:10.1210/er.2009-0006 (2009).

40. Mossa, F. & Ireland, J. J. Physiology and endocrinology symposium: Anti-Müllerian hormone: a biomarker for the ovarian reserve, ovarian function, and fertility in dairy cows. *J Anim Sci* **97**, 1446–1455, doi:10.1093/jas/skz022 (2019).

41. Fleming, R., Seifer, D. B., Frattarelli, J. L. & Ruman, J. Assessing ovarian response: antral follicle count versus anti-Müllerian hormone. Reprod Biomed Online **31**, 486–496, doi:10.1016/j.rbmo.2015.06.015 (2015).
42. Pérez-López, F. R. *et al.* First trimester serum 25-hydroxyvitamin D status and factors related to lower levels in gravids living in the Spanish Mediterranean coast. Reprod Sci **18**, 730–736, doi:10.1177/1933719110396720 (2011).

Figures

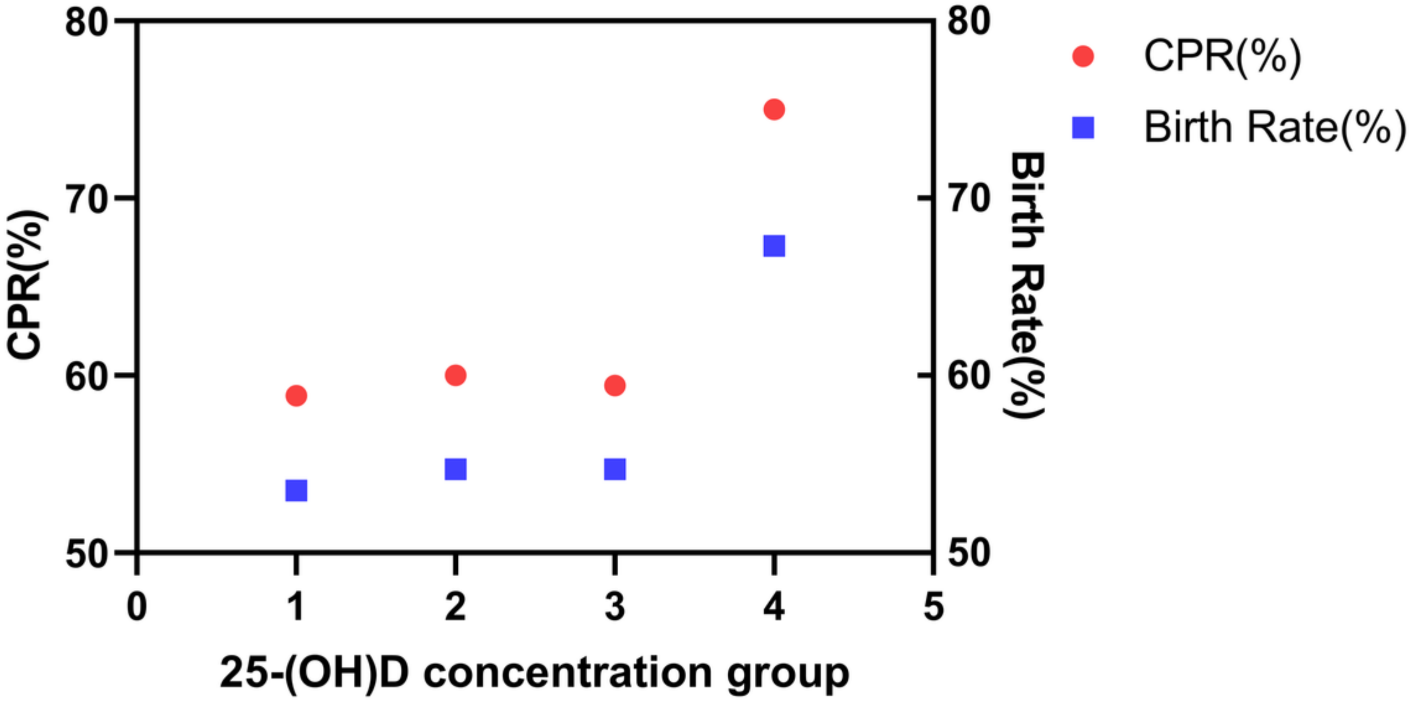

Figure 1

Scatter plot of each 25-(OH)D concentration group and pregnancy outcome. The red dots indicate the relationship between 25-(OH)D concentration and CPR, while the blue squares represent the relationship between 25-(OH)D concentration and BR.

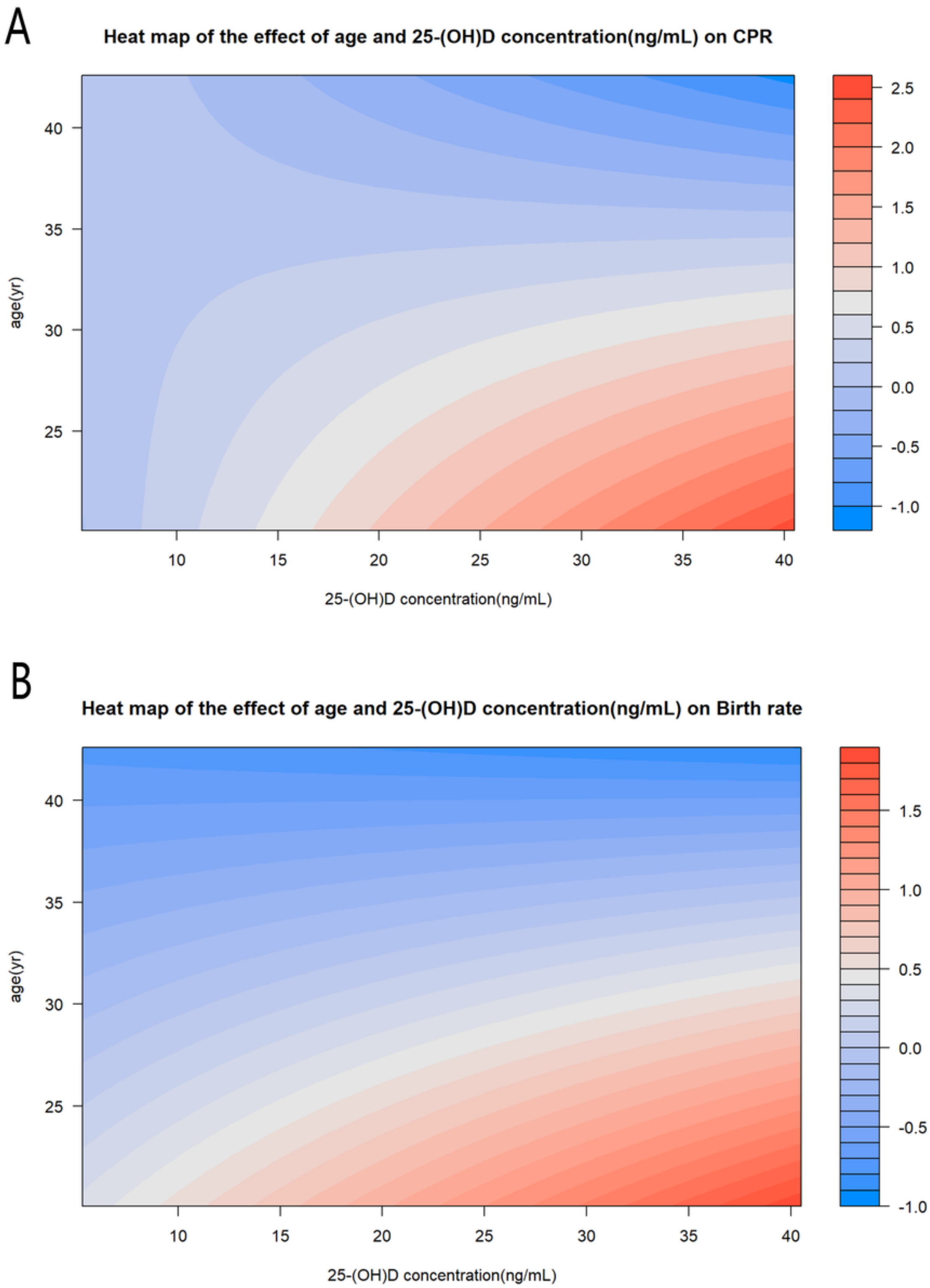

Figure 2

Heat maps for 25-(OH)D concentration (X-axis) and age (Y-axis) corresponding to CPR and BR. Red regions indicate comparatively higher CPR (Fig2.A) as well as BR (Fig2.B) than blue regions. The color was based on the log (odds ratio), shown on the right of the figure.

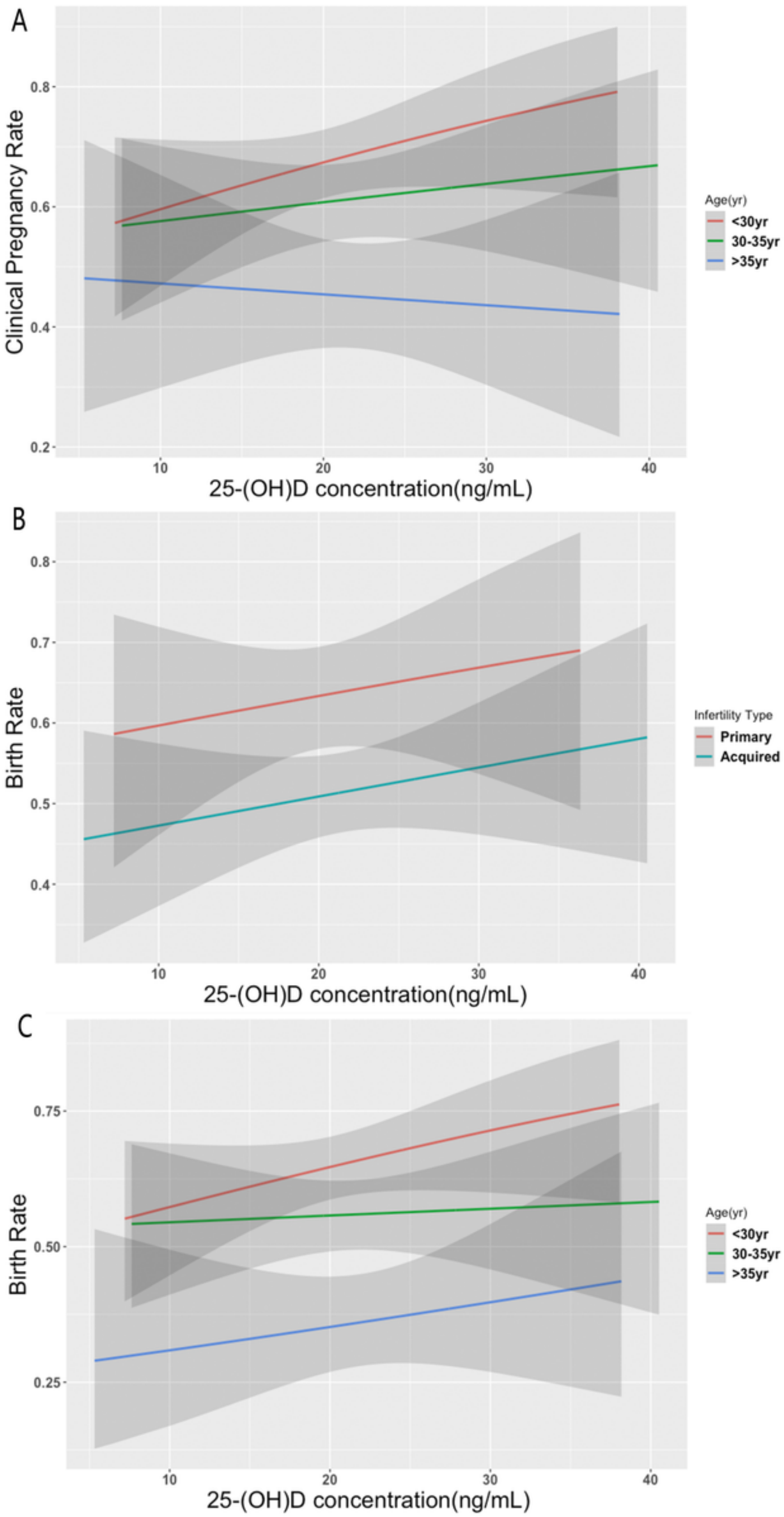

Figure 3

Regression line estimated by logistics regression model with a two-way interaction term between age and 25-(OH)D concentration (Fig3.A and Fig3.C: CPR), and between infertility type and 25-(OH)D concentration (in Fig3.B: BR). The lines indicate estimated clinical pregnancy rate (in Fig3.A) and birth rate (in Fig3.B-C), and the shaded areas represent the 95% confidence intervals. In Fig3.A and Fig3.C, the red line represents women aged younger than 30, the green line indicates women in 30-35 years old and the blue line those older than 35. In Fig3.B, the red line indicates women with primary infertility, while the green line those with secondary infertility.

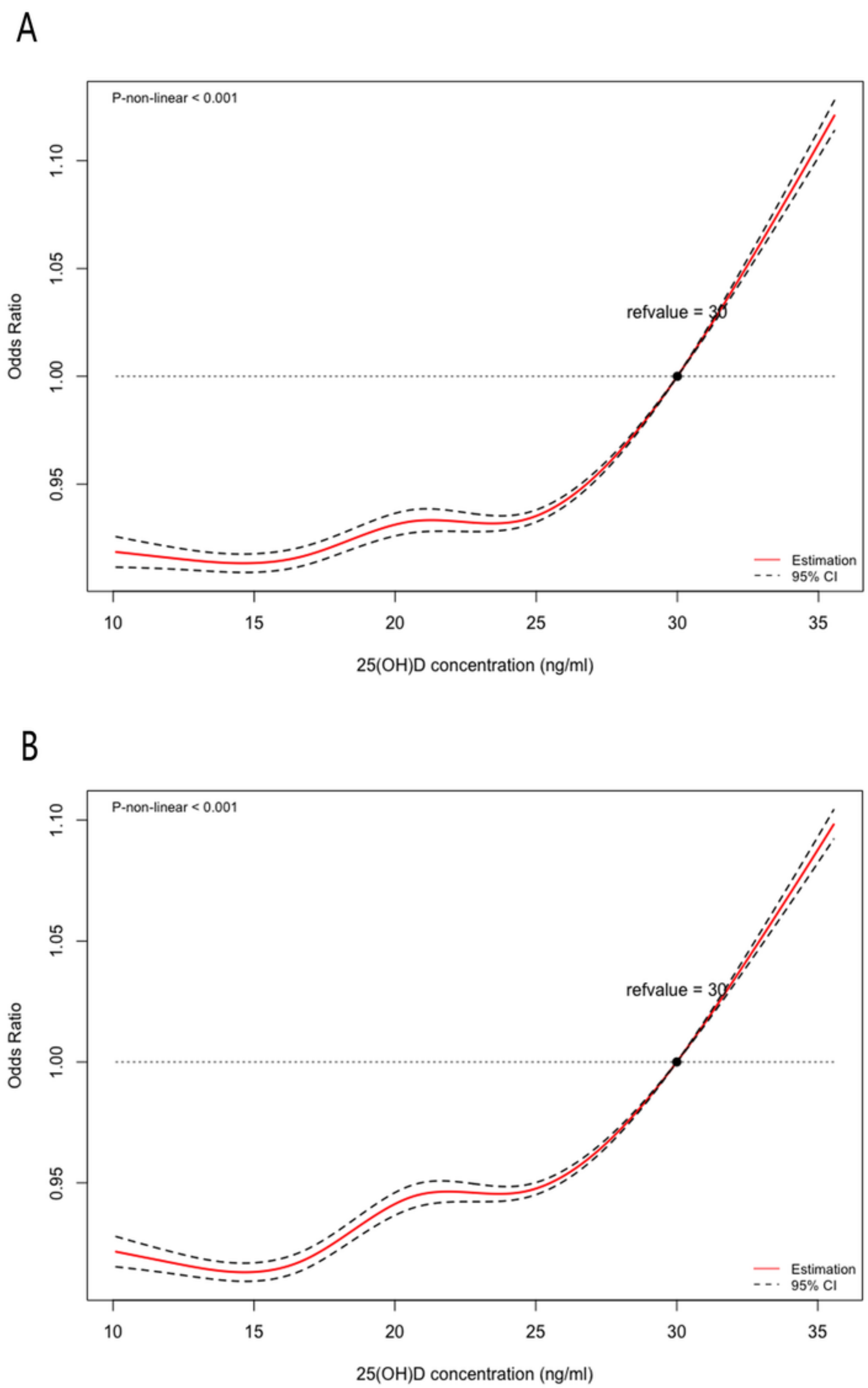

Figure 4

Dose-response relationship between 25(OH)D concentration and predicted Odds Ratio value of clinical pregnancy and birth possibility given by the binary logistic regression model, using restricted cubic spline.

The red solid and black dash lines represent the estimated ORs and their 95% confidence intervals respectively.