Appendix 1:

Summary table of published vitamin D randomized controlled trials conducted in pregnant women in the Middle East and North Africa (MENA) region.

This appendix is adapted from M. Chakhtoura Master of Sciences in Health Research thesis project entitled: "Optimal Dose of Vitamin D Replacement: A systematic Review and Meta-analysis of Randomized Controlled Trials from the Middle East and North Africa", available online from the Jafet Library at the American University of Beirut – Lebanon.

Author Year	City Latitude Country	Sampling method/ setting	Intervention Duration	Nb of subject randomhized per arm	Nb of subject lost to follow up	Age Mean (SD) or median (range) (years)	Primary outcome	Baseline mean (SD) or median (range) 25(OH)D (ng/ml)	Achieved mean (SD) or median (range) 25(OH)D (ng/ml)	Co-morbidities	Compliance (%)	Adverse events
Dawodu JCEM 2013 **NCT02292591**	United Arab Emirates, Al Ain 24.2 ˚N	Primary health care clinics, affiliated with Tawam Hospital	I1: D3 3,600 IU/ d I2: D3 1,600 IU/ d C: Placebo All received also 400 IU daily as prenatal vitamins Duration: 12-16 weeks GA till delivery	I1: 63 I2: 65 C : 64	I1 : 8 I2:13 C : 9	I1: 25.6 (5.5) I2: 27.3 (4.9) C: 27.5 (5.5)	Effectiveness and safety of prenatal vitamin D supplementation	I1:7.8(3.1) I2:8.2(4.8) C: 8.6(5.2)	I1:35.9 (12.1) I2:25.9 (12.2) C :19.3 (19.3)	-	I1:86 I2:87 C :82	None
Shakiba Sing Med J 2013	Yazd, Iran 31.8 ˚N	Two primary care clinics	I1: D3 50,000 IU/month (=1,667 IU/d) I2: 50,000 IU every two weeks (=3,571 IU/d) I3: D3 50,000 IU/week for four weeks, then 50,000 IU/month (=2,579 IU/d) Duration: second trimester until delivery	I1: 17 I2: 17 I3: 17	No lost to follow up	25 (3) (all arms)	Determine the optimal dose of vitamin D necessary during pregnancy in order to attain a vitamin D level > 50 nmol/l in neonates	I1: 16 (7.4) I2: 18 (7.8) I3: 7 (3.0)	In neonates I1: 25 (7) I2: 32 (12) I3: 35 (8) (No maternal results)	-	NA	NA
Soheilykhah Gynecol Endocrinol 2013	Yazd, Iran 31.8 ˚N	Two prenatal clinics (Mojibian Hospital and Shahid Sadoughi Hospital)	I1: D2 200 IU/d I2: D2 50,000 IU/ month (=1,666 IU/d) I3: D2 50,000 IU every 2 weeks (=3,571 IU/d). Duration: 12 weeks GA until delivery	I1: 40 I2: 40 I3: 40	I1: 5 I2: 2 I3: 0	I1: 25 (4.3) I2: 26.5 (4.5) I3: 26.3 (4.8)	Effect of vitamin D supplementation on insulin resistance	I1: 8.3 (7.8) I2: 7.3 (5.3) I3: 7.3 (5.9)	I1: 17.7 (9.3) I2: 27.2 (10.7) I3: 34.1 (11.5)	-	NA	None
Sabet Acta Endocrinol 2012	Tehran, Iran 35.6 ˚N	Mahdieh Hospital	I:D3 100,000 IU/ month (=3,333 IU/d) C: Placebo Duration: 27 weeks GA until delivery	I : 25 C: 25	NA	I : 26.6 (4.7) C: 26 (6.2)	Evaluate the effects on vitamin D supplementation on 25(OJH)D and PTH levels	I : 33.5 (21.4) C: 38.3 (23.2)	Maternal I : 61.4 (30) C: 29.4 (16) Venous cord I : 52 (40.5) C: 26 (21.3)	-	NA	NA
Karamali Horm Metabol Res 2015	Arak, Iran 34.1° N	NA	I: D3 50,000 IU every 14 days (3,571 IU/d) C: Placebo Duration: 3 months	I : 30 C: 30	0	27.4 (5.2) (both arms)	Assess the favorable effects of cholecalciferol administration on metabolic status and pregnancy outcomes in pregnant women at risk for pre-eclampsia.	I: 17 (1.4) C: 17.1(2.2)	I : 34.9 (2.4) C:17.4 (4.0)	At risk for pre-eclampsia	100	NA
Etemadifar Iran J Neurol 2015	Isfahan, Iran 32.6˚N	MS outpatient clinics of Isfahan University of Medical Sciences	I: D3 50,000 IU/ week (=7,142 IU/d) C: Placebo Duration: 12-16 weeks GA till delivery	I : 21 C: 22	I : 15 C: 13	I : 27.7 (2.4) C: 30.0 (3.9)	Assess the safety and efficacy of vitamin D supplementation in multiple sclerosis pregnant women	I : 15.3 (2.9) C: 18.3 (1.9)	I : 33.7 (15.2) C: 14.6 (1.3)	Multiple Sclerosis	Patient who failed to be compliant were excluded	None

NA: Not available; GA: Gestational Age

Appendix 2: Ongoing and completed randomized controlled trials of vitamin D supplementation registered on ClinicalTrials.gov

A-Summary table of ongoing randomized controlled trials of vitamin D supplementation during pregnancy in the Middle East and North Africa (MENA) registered on ClinicalTrials.gov (search was updated in October 2015)

Trial	**Principal Investigator Center Country**	**Estimated number of enrollment (N) Intervention**	**GA at enrollment**	**Outcomes**
1-Vitamin D Supplementation During Pregnancy and Bone Status in Children at Birth and at One Year of Age **NCT01060735** -First received January 2010 -Last updated July 2011 -Last verified July 2011 -Estimated completion date December 2013[1]	Corina Hartman, MD Rabin Medical Center Israel	N=120 Intervention: Vitamin D 2,000 IU/ day Control: Regular supplementation during pregnancy with 400 IU/day vitamin D	27 weeks	Primary Bone status in offspring at the age of one year as assessed by tibial and radius quantitative ultrasound (QUS) Secondary: Vitamin D status of mothers Maternal health status Infant's growth Vitamin D status in infants Safety of vitamin D supplementation doses
2-Vitamin D3 Treatment and Hypocalcemic Pregnant Women **NCT02021864** -First received December 2013 -Last updated January 2014 -Last verified January 2014 -Estimated completion date October 2014[1]	Sima Hashemipour, MD Sina Hospital Iran	N=120 Intervention: Vitamin D3 50,000 IU/week for 8 weeks, daily prenatal multivitamin containing elemental calcium 250 mg/day and D3 400 IU. Control: Daily prenatal multivitamin containing elemental calcium 250 mg/day and vitamin D3 400 IU.	24-26 weeks	Primary: % of hypocalcemic subjects at the end of study Secondary: Changing of maternal mean calcium level

[1] No updates confirming study completion or unknown status

B-Summary table of ongoing randomized controlled trials of vitamin D supplementation during pregnancy worldwide (outside the MENA region) registered on ClinicalTrials.gov (search was updated in October 2015)

Trial	**Principal investigator** **Center** **Country**	**Estimated number of enrollment (N)** **Intervention**	**GA at enrollment**	**Outcomes**
1-Vitamin D Supplementation During Pregnancy for Prevention of Asthma in Childhood (ABCvitaminD) **NCT00856947** -First received March 2009 -Last updated June 2012 -Last verified June 2012 -Estimated completion date April 2014[1]	Hans Bisgaard, MD, DMSc Copenhagen Studies on Asthma in Childhood Denmark	N=600 <u>Intervention:</u> 2,400 IU Vitamin D3 (2 tablets of 1,200 IU) from week 24 of gestation to 1 week after delivery <u>Control:</u> 2 placebo tablets with no active substance, from week 24 of gestation to 1 week after delivery	24-26 weeks	<u>Primary:</u> Recurrent wheeze (0 to 3 years of age) <u>Secondary :</u> Infections (upper / lower respiratory) (0 to 3 years of age) Allergy (0 to 3 years of age) Eczema (0 to 3 years of age) Mothers levels of 25(OH)D, PTH, Calcium, alkaline phosphatase at 1 week after delivery Growth (0 to 3 years of age)
2-Randomized Trial: Maternal Vitamin D Supplementation to Prevent Childhood Asthma (VDAART) **NCT00920621** -First received June 2009 -Last updated August 2013 -Last verified August 2013 -Estimated completion date June 2014[1]	Scott T Weiss Brigham and Women's Hospital US	N=870 <u>Intervention:</u> 4,000 IU of vitamin D3 administered orally once a day <u>Control:</u> Placebo	10-18 weeks	<u>Primary:</u> Asthma or recurrent wheeze in the child (1-3 years) <u>Secondary:</u> Allergic sensitization (total and specific IgE) Eosinophil count Doctor's diagnosis of eczema Lower respiratory tract infections (3 years) Children's levels of 25OHD (1-3 years) Preterm birth (birth <37 weeks gestation), preeclampsia, gestational hypertension, and/or hemolytic anemia, elevated liver enzymes, low platelet count (HELLP syndrome)

Trial	**Principal investigator Center Country**	**Estimated number of enrollment (N) Intervention**	**GA at enrollment**	**Outcomes**
3-Effects of Vitamin D Supplement Before and During Pregnancy on Birth Weight (Gravita) **NCT01038453** -First received December 2009 -Last updated November 2011 -Last verified November 2011 -Estimated completion date December 2011[1]	Gitte Bloch Rasmussen, MD Aarhus University Hospital Denmark	N=400 <u>Intervention 1:</u> Vitamin D3 oral 1 tablet 35 µg per day. Placebo oral 1 tablet per day. In total 35 µg per day (1400 IU) <u>Intervention 2:</u> Vitamin D3 oral 2 tablets each containing 35 µg. In total 70 µg per day (2,800 IU) <u>Control:</u> Placebo 2 tablet, once a day	Enrollment date not mentioned[3]	<u>Primary</u> Birth weight <u>Secondary</u> Post-partum effects of vitamin D supplement on maternal bone mineral density (BMD) (1 to 4 months post-partum) Infections of the newborn (Day 1 of the child to 16 weeks after birth) Growth of the newborn measured by weight, crown-heel length and head circumference Time to accomplish pregnancy (0 to 12 months)
4-Prevention of Adverse Pregnancy Outcome With Vitamin D Supplementation During Pregnancy **NCT01418664** -First received August 2011 -Last updated August 2011 -Last verified August 2011 -Estimated completion date March 2012[1]	Nazli Hossain, MBBS, FCPS University of Health Sciences Pakistan	N=200 <u>Intervention:</u> Routine ferrous sulphate and calcium lactate, 4,000IU of vitamin D <u>Control:</u> Ferrous sulphate and calcium lactate only	Not mentioned; eligible women < 20 weeks gestation	<u>Primary:</u> Prevention of adverse pregnancy outcome, preeclampsia, small for gestational age (SGA), preterm labor, at 18 months <u>Secondary:</u> Cord levels and maternal serum levels of $1,25(OH)_2D$, after supplementation at the time of delivery, at 18 months

Trial	Principal investigator Center Country	Estimated number of enrollment (N) Intervention	GA at enrollment	Outcomes
5-Effect of Calcium Plus Vitamin D Supplementation on Adolescent Mother and Infant Bone Health **NCT01732328** -First received November 2012 -Last updated November 2012 -Last verified November 2012 -Estimated completion date June 2013[1]	Flavia F Bezerra, DSc Universidade do Estado do Rio de Janeiro Brazil	N=76 <u>Intervention:</u> Calcium plus vitamin D: 600mg of calcium and 200 IU vitamin D daily <u>Control:</u> Inactive pill (microcrystalline cellulose and corn starch) taken daily	23-29 weeks	<u>Primary:</u> Differences in maternal bone mass changes postpartum between supplemented and placebo groups (2, 5 and 12 months postpartum) Differences in fetal growth and infant bone mass between supplemented and placebo groups <u>Secondary:</u> Differences in changes of bone and calcium related hormones and vitamin D status between supplemented and placebo groups Differences in human breast milk composition (nutrients and hormones) between supplemented and placebo groups Differences in maternal bone status according to vitamin D receptor (VDR) polymorphisms Differences in infant body composition between supplemented and placebo groups Differences in changes of maternal body composition postpartum between supplemented and placebo groups
6-Vitamin D Status Impacts Inflammation and Risk of Infections During Pregnancy **NCT01815047** -First received March 2013 -Last updated May 2015 -Last verified May 2015 -Estimated completion date June 2016	Kimberly O'Brien, PhD Cornell University US	N=158 <u>Intervention 1:</u> Vitamin D3 400 IU daily. <u>Intervention 2:</u> Vitamin D 3 4,000 IU daily	Around 26 weeks	<u>Primary:</u> Change in maternal calciotropic hormones (25(OH)D, $1,25(OH)_2D$ and PTH) and inflammatory cytokines (CRP, interleukin (IL) -6 and IL-10 and tumor necrosis factor (TNF) - alpha) (taken at baseline, 23-28 weeks GA and at delivery) <u>Secondary:</u> Retrospective analysis: Change in maternal calciotropic hormones (25(OH)D, 1,25(OH)2D and PTH and inflammatory cytokines (CRP, IL-6 and IL-10 and TNF-alpha) in the archived serum collected from a cohort of 168 adolescents that were longitudinally followed across pregnancy both at mid-gestation and at delivery

Trial	**Principal investigator** **Center** **Country**	**Estimated number of enrollment (N)** **Intervention**	**GA at enrollment**	**Outcomes**
7-Maternal Vitamin D for Infant Growth (MDIG) Trial **NCT01924013** -First received August 2013 -Last updated August 2013 -Last verified August 2013 -Estimated completion date April 2016	Daniel Roth, MD The Hospital for Sick Children Bangladesh	N=1300 <u>Intervention 1:</u> Prenatal Period 4,200 IU/week of vitamin D3 (=600 IU/d); Postpartum Period: placebo <u>Intervention 2:</u> Prenatal Period 16,800 IU/week of vitamin D3 (=2,400 IU/d); Postpartum Period: placebo <u>Intervention 3:</u> Prenatal Period 28,000 IU/week of vitamin D3 (=4,000 IU/d); Postpartum Period: placebo <u>Intervention 4:</u> Prenatal Period 28,000 IU/week of vitamin D3 (=4,000 IU/d); Postpartum Period: 28,000 IU/week (=4,000 IU/d) <u>Control:</u> Placebo	17-24 weeks	<u>Primary:</u> Infant Length-for-Age Z-Scores (LAZ) with Prenatal Supplementation Infant LAZ with Postpartum Supplementation <u>Secondary:</u> Serum calcium <u>Others:</u> Stunting LAZ < -2 SD below the median) at 1 and 2 years of age Attained length and LAZ at 2 years of age. Birth weight, low birth weight % Small for gestational age (SGA) % Preterm birth % Stillbirth %
8-Preventing Health Disparities During Pregnancy Through Vitamin D Supplementation **NCT01932788** -First received August 2013 -Last updated NA -Last verified May 2013 -Estimated completion date April 2016	Carol L Wagner, MD Medical University of South Carolina US	N=450 <u>Intervention:</u> 4000 IU/day vitamin D3 in gummy vitamin form, plus the standard prenatal vitamin (containing 400 IU vitamin D3, <u>Control:</u> Placebo gummy, plus the standard prenatal vitamin (containing 400 IU vitamin D3)	10-14 weeks	<u>Primary:</u> Change in 25(OH)D from baseline to delivery (maternal and infant) No other outcomes mentioned although they stated that the purpose of this study is to examine the effectiveness and infection-fighting properties of the body in relationship to vitamin D levels

Trial	**Principal investigator Center Country**	**Estimated number of enrollment (N) Intervention**	**GA at enrollment**	**Outcomes**
9-Prospects for the Prevention of Pregnancy-induced Hypertension and Preeclampsia Trial (4P) **NCT02007837** First received -November 2013 Last updated -December 2013 Last verified -December 2013 Estimated completion date -February 2017	Diederick E Grobbee, MD PhD UMC Utrecht Ghana Health Services University of Ghana Ghana	N=440 <u>Intervention:</u> In a single capsule, the following will be combined: 80 mg low-dose aspirin, 1.2 grams calcium, 600 IU vitamin D, 5mg folic acid and 1000 ug vitamin B12 <u>Control:</u> Placebo, 5mg folic acid, cellulose filler	GA at enrollment not mentioned[4]	<u>Primary:</u> Development of pregnancy-induced hypertension in pregnancy. Development of a de novo systolic blood pressure of > 140 mmHg, diastolic blood pressure of >90 mmHg, measured at least twice <u>Secondary:</u> Maternal/obstetric outcomes: maternal death, preeclampsia, eclampsia, hemolysis, HELLP syndrome, hemorrhage, caesarian section, other complications during pregnancy or delivery Neonatal and infant outcomes: preterm birth, intra uterine death, stillbirth, neonatal mortality, congenital abnormality, neonatal intensive care unit admission or pediatrician referral, birth weight, SGA, APGAR scores, other adverse effects. Infant outcomes: weight and height, health, occurrence of disease and general health status Number of participants with (severe) adverse events as a measure of safety and tolerability
10-Assessment of Dose Effectiveness of Vitamin D Supplementation During Pregnancy- a Dose Comparison Trial **NCT02215213** -First received August 2014 -Last updated June 2015 -Last verified June 2015 -Estimated completion date July 2015[2]	Sidrah Nausheen, FCPS Aga Khan University Pakistan	N=315 <u>Intervention 1:</u> Vitamin D3 2,000 IU daily <u>Intervention 2:</u> Vitamin D3 4,000 IU daily <u>Intervention 3:</u> Vitamin D 3 400 IU daily	Not mentioned. Eligible women < 16 weeks gestation	<u>Primary:</u> Pregnancy out comes at delivery: Hypovitaminosis, pre-eclampsia, preterm labour, preterm birth, low birth weight, still birth rates <u>Secondary:</u> Prevalence of vitamin D deficiency in pregnant women at recruitment

Trial	**Principal investigator Center Country**	**Estimated number of enrollment (N) Intervention**	**GA at enrollment**	**Outcomes**
11-Is Vitamin D Insufficiency and Deficiency Associated With Antepartum and Postpartum Depression? **NCT02272387** -First received October 2014 -Last updated June 2015 -Last verified June 2015 -Estimated completion date July 2017	Principal investigator name not mentioned St. Luke's-Roosevelt Hospital Center US	N=750 <u>Intervention 1:</u> Vitamin D3 50,000 IU tablet weekly x 8 weeks plus prenatal vitamin (400 IU vitamin D) <u>Control:</u> Placebo tablet plus prenatal vitamin D3 400 IU	< 20 weeks	<u>Primary:</u> Antepartum and postpartum depressive symptoms at 9 months <u>Secondary:</u> Maternal morbidities (antepartum and delivery) Composite maternal complications: preeclampsia, GDM, delivery complications, chorioamnionitis. Fetal morbidities (antepartum and delivery) Composite outcomes: SGA, intra-uterine growth retardation, low APGAR, low cord gases, hydramnios
12-Maternal Vitamin D for Acute Respiratory Infections in Infancy (MDARI) **NCT02388516** -First received March 2015 -Last updated September 2015 -Last verified September 2015 -Estimated completion date August 2016	Shaun K Morris, MD, MPH Daniel Roth, MD, PhD The Hospital for Sick Children. US Trial conducted in Bangladesh	N= 1300 <u>Intervention 1:</u> Prenatal Period vitamin D3 4,200 IU/week; Postpartum period (for 6 months): placebo <u>Intervention 2:</u> Prenatal period vitamin D3 16,800 IU/week; Postpartum period: placebo <u>Intervention 3:</u> Prenatal period vitamin D3 28,000 IU/week; Postpartum period: 28,000 IU/week <u>Control:</u> Placebo	17-24 weeks	<u>Primary:</u> Microbiologically confirmed viral acute respiratory infection at 0 to 6 months of age <u>Secondary (all at 0 to 6 months of age):</u> Acute respiratory infection with microbiologically confirmed influenza A or B. Acute respiratory infection with microbiologically confirmed RSV. Clinical upper and/or lower respiratory tract infection (i.e., no microbiological confirmation) Clinical upper respiratory tract infection Clinical lower respiratory tract infection Quantitative S. pneumoniae nasal carriage density Pneumonia (non-severe) and severe pneumonia or very severe disease

Trial	**Principal investigator Center Country**	**Estimated number of enrollment (N) Intervention**	**GA at enrollment**	**Outcomes**
13-Trial of Vitamin D Supplements to Raise Calcidiol Levels of Pregnant Women in Mongolia **NCT02395081** First received February 2015 Last updated March 2015 Last verified March 2015 Estimated completion date July 2017	Janet Rich-Edwards, ScD Brigham and Women's Hospital, US trial conducted in Mongolia	N=360 <u>Intervention 1:</u> Vitamin D3 600 IU daily <u>Intervention 2:</u> Vitamin D3 2,000 IU daily <u>Intervention 3:</u> Vitamin D3 4,000 IU daily	12-16 weeks	<u>Primary:</u> Circulating 25(OH)D serum levels at delivery <u>Secondary:</u> Preeclampsia prevalence as measured by new onset hypertension after 20 weeks gestation and proteinuria Average monthly change in blood pressure as measured Arterial tonometry at 36 weeks GA Preterm delivery as measured by clinical diagnosis Caesarean section (from the medical record) Assisted vaginal delivery (from the medical record) Small or large for gestational age neonate as measured by medical record abstraction, at delivery Calcemia as measured by serum calcium test Proteinuria, measured by urine dipstick at 36- 40 weeks GA Hypertensive disorders of pregnancy prevalence as measured by clinical diagnosis Bacterial vaginosis
14-Nutritional Requirements for Vitamin D in Pregnant Women (DMAT) **NCT02506439** -First received May 2015 -Last updated July 2015 -Last verified July 2015 -Estimated completion date: December 2016	Mairead Kiely, PhD Louise Kenny, PhD University College Cork Ireland	N= 192 <u>White skinned women:</u> <u>Intervention 1:</u> Vitamin D3 400 IU daily <u>Intervention 2:</u> Vitamin D3 800 IU daily <u>Control:</u> Placebo <u>Black skinned women</u>: All receive vitamin D3 800 IU daily.	15 weeks	<u>Primary:</u> Serum 25(OH)D in pregnant women and cord blood <u>Secondary:</u> Serum total calcium Maternal blood pressure during pregnancy Serum Parathyroid Hormone

[1] No updates confirming study completion or unknown status.

[2] Ongoing but not recruiting.

[3] Women who do not become pregnant during the first 12 months of treatment would be excluded from further participation in the study (personal communication with the principal investigator).

[4] Women at any GA below 16 weeks are eligible (personal communication with the principal investigator).

C-Summary table of completed (unpublished) randomized controlled trials of vitamin D supplementation during pregnancy registered on ClinicalTrials.gov (search was done in December 2015)

Trial	**Principal investigator Center Country**	**Estimated number of enrollment (N) Intervention**	**GA at enrollment**	**Outcomes**
1-Effects of Vitamin D Supplementation During Pregnancy on Clinical Outcomes and Immune Function. **NCT01417351** -First received August 2011 -Last updated July 2013 -Last verified July 2013	Charles B Stephensen, PhD USDA, Western Human Nutrition Research Center, California USA	N=60 <u>Intervention 1:</u> 400 IU Vitamin D3 daily <u>Intervention 2:</u> 2,000 IU vitamin D3 daily	<20 weeks	<u>Primary:</u> Change in T-cell cytokine expression at three time points (6-20 weeks, 26-28 weeks, 36 weeks gestation) <u>Secondary</u> at 6-20 weeks, 26-28 weeks, 36 weeks gestation): Change in vitamin D status (25(OH)D and $1,25(OH)_2D$ levels) Change in innate immune function Change in blood pressure
2-Study of Vitamin D Supplementation on Improvement of Gums Health **NCT01422122** -First received August 2011 -Last updated August 2012 -Last verified August 2012	Zulfiqar A Bhutta, FRCPCH, PhD Aga Khan University, Pakistan	N=115 <u>Intervention:</u> 4,000 IU of vitamin D3 administered orally once a day (one tablespoon syrup per day) <u>Control:</u> Placebo	12-20 weeks	<u>Primary:</u> Periodontal Probing Depth at 6 months <u>Secondary:</u> IL-6 levels at 6 months

Abbreviations:
25(OH)D: 25-Hydroxyvitamin D; 1,25(OH)2D: 1,25-Dihydroxyvitamin D; HELLP: Hemolytic anemia, elevated liver enzymes, low platelet count; IL: Interleukin; LAZ: Length-for-Age Z-Scores; SGA: Small for gestational age; TNF: Tumor necrosis factor; QUS: Quantitative ultrasound.

Appendix 3: Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)

This appendix includes details on specific items of the SPIRIT checklist that were not described in the main protocol manuscript

Administrative information

1-Title:
A multicenter, blinded, randomized parallel group superiority study to compare the efficacy of different equivalent daily doses of vitamin D: 600 IU vs 3,000 IU in pregnant women with vitamin D insufficiency.

2-Trial registration:
Table 1: World Health Organization Trial Registration Data Set

Data category	**Information**
Primary Registry and Trial Identifying Number	ClinicalTrials.Gov NCT 2434380
Date of Registration in Primary Registry	April 2015.
Secondary Identifying Numbers	IM.GE-HF.22.
Source(s) of Monetary or Material Support	Medical Practice Plan Research Funding at the American University of Beirut, Beirut, Lebanon. WHO - Eastern Mediterranean Regional Office Special Grant for Research in Priority Areas of Public Health, 2014-2015.
Primary Sponsor	American University of Beirut-Medical Center (AUB-MC).
Secondary Sponsor(s)	Not applicable.
Contact for Public Queries	bmd@aub.edu.lb
Contact for Scientific Queries	gf01@aub.edu.lb
Public Title	Vitamin D replacement in pregnant women with Vitamin D insufficiency: a randomized controlled trial.
Scientific Title	A three center, blinded, randomized parallel group, vitamin D superiority trial to compare the efficacy of equivalent daily doses of 3000 IU versus 600 IU daily in pregnant women, on maternal and neonatal outcomes in pregnant women with vitamin D insufficiency.
Countries of Recruitment	Lebanon.
Health Condition(s) or Problem(s) Studied	Vitamin D insufficiency during pregnancy.
Intervention(s)	Equivalent of daily Vitamin D, at one of two doses: Low dose : 600 IU. High dose: 3,000 IU.
Key Inclusion and Exclusion Criteria	Inclusion criteria: • Pregnant women gestational age (GA) < 14 weeks at screening visit.

	• Middle Eastern origin: Middle East countries as defined by WHO (Bahrain, Egypt, Iran, Iraq, Palestine, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, Syria, United Arab Emirates, Yemen). • 25(OH)D level between 25 and 75 nmol/l. • Age > 18 years. • Vitamin D supplementation ≤ 200 IU daily. <u>Exclusion criteria:</u> • 25(OH)D level < 25 nmol/l, as it would be unethical to give low doses of vitamin D supplementation in this situation, and 25(OH)D level > 75 nmol/l, as vitamin D supplementation with routine prenatal multivitamins would be enough. • Known metabolic bone disease or chronic diseases associated with bone abnormalities (renal or liver diseases). • Current medications likely to interfere with vitamin D metabolism (enzyme inducing anticonvulsants, anti-tuberculosis). • Vitamin D supplementation> 600 IU daily*. • Fetal physical anomalies on the initial ultrasound. • Renal stones. • Hyperparathyroidism. • Uncontrolled thyroid dysfunction. • Diagnosis of cancer in the last 10 years (other than basal cell carcinoma). • Serum calcium >10 mg/dl. • Diabetes mellitus type 1 or type 2. • Previous gestational diabetes mellitus. • Allergy to any component of vitamin D formulation. *If a pregnant woman is on a high dose of vitamin D supplementation, > 600 IU daily, vitamin D should be stopped at least one month prior to study entry, at the discretion of her primary physician.
Study Type	Phase III, Randomized controlled, superiority trial with two parallel groups.
Date of First Enrollment	July 27, 2015
Target Sample Size	280 pregnant women.
Recruitment Status	Ongoing
Primary Outcome(s)	• The proportion of women who will reach the Institute Of Medicine (IOM) defined desirable 25(OH)D level ≥ 50 nmol/l at delivery. • The infant bone mineral content (BMC) at one month of age.
Key Secondary Outcomes	• Maternal outcomes: Mean maternal 25(OH)D level, at delivery. Mean maternal PTH level, at delivery. Mean change in maternal urine calcium. • Neonatal outcomes: Mean neonatal 25(OH)D level, at birth. Mean neonatal PTH level at birth. Mean neonatal fat mass, at one month of age. Mean neonatal knee to heel length at birth

Protocol version

3-Date and version identifier
December 2015; Version 7

4-Funding:
Partial funding from:
1-Medical Practice Plan Research Funding at the American University of Beirut, Beirut, Lebanon.
2-WHO - Eastern Mediterranean Regional Office Special Grant for Research in Priority Areas of Public Health, 2014-2015.

Roles and responsibilities
5a-Names, affiliations, and roles of protocol contributors:
-Ghada El Hajj Fuleihan, MD, MPH.
Professor of Medicine.
American University of Beirut, Lebanon.
Role: Principal investigator is responsible for study conception, study design, proposal development, protocol update(s) and revision(s), subject recruitment, oversight of data collection, data entry, data review, data analysis, interpretation, and review of manuscript drafts, revision and approval of the final versions submitted for publication.

-Marlene Chakhtoura, MD, MSc.
Senior Research Fellow.
American University of Beirut, Lebanon.
Role: Co-investigator, is responsible for proposal development, protocol update(s) and revision(s), subject recruitment, data entry, analysis and interpretation, and review of manuscript drafts and approval of the final version submitted for publication.

-Anwar Nassar, MD.
Professor of Obstetrics and Gynecology.
American University of Beirut, Lebanon.
Role: Co-investigator, is involved in proposal revision, subject recruitment, review of data analysis and interpretation, manuscript drafts and approval of the final versions.

-Ziyad Mahfoud, PhD.
Associate Professor of Medicine.
Weil Cornell, Qatar.
Consultant American University of Beirut, Lebanon.
Role: Co-investigator, serves as trial statistician and provides advice on some aspects of clinical trial design (randomization methodology), review of sample size calculation and analysis plan, oversight of analyses with the principal investigator and the research fellow, and approval of the final version of manuscript.

-Cyrus Cooper, MD, FRCP.
Professor of Medicine.
University of Southampton, UK.
and
-Nicholas Harvey, MA, MB, BChir, MRCP, PhD.
Professor of Medicine.
University of Southampton, UK.

Role: Co-investigators, are responsible for sharing their expertise in conducting a clinical trial in pregnancy, sharing trial documents, consent form, data collection sheets, contributing to proposal review, and revision, data interpretation, review of manuscript drafts and approval of the final versions.

-Asma Arabi, MD, MSc.
Associate Professor of Medicine.
American University of Beirut, Lebanon.
Role: Co-investigator, is involved in trial daily operations oversight and member of Trial Steering Committee (TSC).

-Mona Nabulsi, MD, MSc.
Professor of Clinical Pediatrics.
American University of Beirut, Lebanon.
Role: Collaborator, is involved in nursery and neonatal assessment and member of TSC.

-Lama Charafeddine, MD, FAAP.
Associate Professor of Clinical Pediatrics.
American University of Beirut, Lebanon.
Role: Collaborator, is involved in nursery and neonatal assessment.

5b-Name and contact information for the trial sponsor:
Trial Sponsor: American University of Beirut-Medical Center (AUB-MC)
Address: Hamra-Beirut
Lebanon
Telephone : 01-350 000
Email : aubmc@aub.edu.lb

5c-Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities.
AUB-MC provides the infrastructure for the trial conduct, including the Office of Grants and Contracts, the Biomedical Institutional Review Board, and the Clinical Research Unit, where the trial will take place. The sponsor and the funding source have no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

WHO - Eastern Mediterranean Regional Office Special Grant for Research in Priority Areas of Public Health, 2014-2015, provided partial funding to initiate the trial conduct, and cover basic studies and assays.

5d-Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable

Trial Steering Committee (TSC):
Dr Ghada El Hajj Fuleihan.
Dr Marlene Chakhtoura.
Dr Anwar Nassar.
Dr Asma Arabi.
Dr Ziyad Mahfoud.
Dr Mona Nabulsi.
Dr Cyrus Cooper.
Dr Nicholas Harvey.

Role:
-Study planning.
-Agreement on final protocol, reviewing progress of study and if necessary agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study.

Trial Monitoring Committee:
-Dr Ghada El Hajj Fuleihan.
-Dr Marlene Chakhtoura.
-Dr Anwar Nassar.
-Dr Mona Nabulsi.
-Dr Asma Arabi.

Role:
-Overall oversight of trial operations.
-Verification of case report forms (CRFs).
-Oversight of data collection, entry and verification.
-Quality assurance and compliance with protocol stipulations.
-Evaluation of all adverse events (AEs) and Serious Unexpected AEs.
-SUSAR (Serious Unexpected Suspected Adverse Events) reporting.
-Budget administration and contractual issues with individual centers.
-Responsible for trial master file (Drs El Hajj Fuleihan and Chakhtoura).

Data safety and monitoring board (DSMB):
Dr Christopher Gallagher, Department of Endocrinology, Creighton University Omaha, Nebraska.
Dr Munro Peacock, Department of Medicine, Indiana University, Indianapolis.

Dr Michael Holick, Department of Medicine, Boston University Medical Center, Boston.

Role:
-Review of all serious AEs.
-Makes recommendations concerning the unblinding of data for DSMB oversight, and makes recommendations on re-continuation, modification, or termination of the trial.

Introduction

6a-Background and rationale
See main protocol manuscript.

6b-Explanation for choice of comparators:

Comparators: Two arms of different daily doses of vitamin D supplementation:

- Low dose : 600 IU daily
- High dose: 3,000 IU daily

Given the high prevalence of low vitamin D levels in the Lebanese population, it would be unethical to have a placebo group. The lowest dose is selected as the dose approximating the RDA as defined by the IOM.

The highest dose is slightly lower than the upper limit of intake (4,000 IU daily) and this is the dose that is projected to allow to 97.5% of the population to reach a 25(OH)D >50 nmol/l. This projection is based on median baseline 25-OHD levels in Lebanese women of reproductive age and the anticipated increments in response to supplementation based on the conservative estimate of 0.7 ng/ml increase in 25(OH)D for each 100 IU of additional vitamin D intake, as detailed in sample size calculation.

7-Objectives:
See main manuscript.

8-Study design:
See main manuscript.

Methods: Participants, interventions and outcomes

9-Study setting:
See main manuscript.

10- Eligibility criteria:
See main manuscript.

11-Interventions:

a- Description:
All participants will be given vitamin D supplementation different dose tablets but with similar shape, color and consistency. Europharm is the drug company that will provide us with the intervention drug.

The low dose arm will be given an equivalent dose of 600 IU vitamin D daily. The high dose arm will be given an equivalent dose of 3,000 IU vitamin D daily.
We will consider administering 10,000 IU vitamin D tablets and placebo tablets on weekly basis.
➔low dose arm group receives 10,000 IU vitamin D tablet and 1 placebo tablet every other week, alternating with 2 placebo tablets every other week (daily dose would be 10,000/14=714 IU).
➔High dose arm groups receives 2 tablets 10,000 IU vitamin D weekly (daily dose would be 2,857 IU).
Vitamin D supplementation will be started within 4-6 weeks of initial visit, at 15-18 weeks GA, after study subjects would have signed consent form and completed all baseline studies.

Vitamin D supplementation as part of prenatal vitamins is allowed at dose not exceeding 200 IU daily. Accordingly, the study groups will receive the following does:

- Low dose 714+200=914 IU daily
- High dose 2,857+200=3,057 IU daily

b- Stopping rules:

Interim analysis will not be done; previous studies on similar, even higher doses of vitamin D supplementation during pregnancy [1,2] did not result in any reported cases of vitamin D toxicity. Furthermore, no early beneficial effects leading to early termination of the study are expected.

Monitoring for adverse events:
-25(OH)D level >250* nmol/l at any time, with concomitant hypercalcemia.
-Development of kidney stones or hypercalcemia.
-Increments in serum calcium, or serum creatinine will be managed as per attached algorithm
(* Vitamin D intoxication occurs at a level >375 nmol/l [3], we are considering a lower cutoff to be more conservative).
(See Appendix 4)

c- Adherence:

Phone call every two weeks by the study team will be done to enhance compliance. Such approach ensures 90% adherence, as demonstrated in our previous vitamin D randomized controlled trial conducted in elderly Lebanese men and women [4]. Weekly drug administration, as opposed to daily regimen, would also improve compliance.
Face to face reminder of the instructions related to vitamin D administration will occur at each trial visit.
Pill count will also be implemented at each visit to check for compliance.

d- Concomitant care and interventions:

The standard care of other vitamins and mineral supplementation during pregnancy will be allowed including 1 or 2 tablets of 500 mg of calcium daily, depending on dietary intake, and a vitamin D dose not exceeding 200 IU daily.

12-Outcomes:

See main manuscript.

13-Participant timeline

	Study period					
	Study visits					
	Prescreening	Screening	Enrollment and Allocation	Follow up	Follow up	Close out
Time point	-t2 1st trimester	-t1 11-13 week	t0 15-18 weeks	t1 28-32 weeks	t2 37-41 weeks (delivery)	t3 1 month post-partum
Enrollment:						
-Eligibility screen	×	×	×			
-Informed consent	×					
Allocation			×			
Interventions						
Low dose 600 IU/d			←	—	→	
High dose 3,000 IU/d			←	—	→	
Assessments			Interview	Interview	Interview	Interview
			Maternal height, weight and blood pressure	Maternal weight and blood pressure	Maternal weight and blood pressure. Paternal height. Neonatal anthropometric studies	
		Blood and urine tests: 25(OH)D, 1,25(OH)D, PTH, Crea, Ca, Ph, Mg, alb, TSH, 24h (or spot) urine for Ca & crea		Blood and urines tests: 25(OH)D, Crea, Ca, 24h (or spot) urine for Ca & crea	Blood tests: 25(OH)D, Crea, Ca, placental and genetic and placental studies	Infant BMD

See main manuscript for further details.

14-Sample size calculation:

See main manuscript.

15- Recruitment:

See main manuscript.

Methods: Assignment of interventions

16-Assignement of interventions:

a-Allocation—sequence generation:

Participants will be randomized to one of the two arms of vitamin D supplementation, 600 IU or 3,000 IU daily in a 1:1. The statistician will generate a randomization sequence using an online randomization generator (www.randomization.com). Randomization will be stratified by center, using restricted permuted block randomization. Blocks details (including number of blocks in each arm and number of participants in each block) will not be disclosed. A separate document describing full randomization information will be provided, with restricted access – only to the pharmacy.

b- Allocation—concealment mechanism:

The pharmacy provides the treatment allocation, and prepares study drug boxes containing study drug pills that look alike regardless of treatment allocation; all participants will take 2 pills every week that would have been pre-packed in boxes (boxes are divided in cubicles; each cubicle has a label indicating the week when the pills should be taken).

c- Allocation—implementation:

The statistician is responsible of sequence generation and treatment assignment. The pharmacist is responsible for treatment allocation. The research assistant is responsible of participant enrollment and intervention drug delivery to each participant.

After an eligible pregnant woman consents to participate in the trial and is enrolled, an email will be sent to the pharmacist to prepare study drug. Study drug is picked up by the research assistant who delivers it to the participant.

17- Blinding (masking):

17a- Blinding (masking):

Trial participants, care providers, data collectors, outcome assessors and data analysts will be blinded. Only the DSMB will have access to unblinded data. Blinding of trial participants will be ensured using vitamin D supplementation using vitamin D or placebo pills of similar shape, color, smell and consistency. Unblinding of care providers, data collectors, outcome assessors and data analysts will be further reduced by assigning for each participant a specific unique code (instead of a fixed code for all participant of the same group), so that all information and results will be coded.

17-b Blinding (masking)—emergency unblinding

Emergency unblinding will be done if the participant develops serious adverse event that is considered by DSMB to be most likely related to the intervention drug.

The health care professional or research assistant should inform the investigator of the reasons for code break as soon as possible. The Investigator must report all code breaks (with reason) as they occur on the corresponding CRF and inform DSMB, IRB and the pharmacy about it. It should also be mentioned in the participant Medical Record.

Methods: Data collection, management and analysis

18-Data collection methods

a-Data collection methods, management, and analysis

A-Laboratory measurements:

All routine chemistry tests including serum lipids, calcium, phosphorus and creatinine were measured at AUB-MC using Roche Cobas 6000 auto-analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Screening 25(OH)D level will be performed locally using Electrochemiluminescence Immunoassay (ECLIA). All calciotropic hormones including 25(OH)D levels will be run using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Serum Calcium: is measured by 5-nitro-5'-methyl-BAPTA (NM-BAPTA) using Roche Cobas 6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Inter-assay CVs are 1.6% and 1.5% at 9.3 and 12.1 mg/dl, respectively.

Serum Creatinine: is measured by the enzymatic assay using Roche Cobas 6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Inter-assay CVs are 2.3%, 1.7%, and 1.5% at 1.0, 2.0, and 5.4 mg/dl, respectively.

Serum Phosphorus: is measured by ammonium molybdate method using Roche Cobas 6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Inter-assay CVs are 1.7% and 1.6% at 3.6 and 7.4 mg/dl, respectively.

25 Hydroxyvitamin D2: using LC-MS (ThermoFisher Scientific, Franklin, Massachusetts 02038 and Applied Biosystems-MDS Sciex, Foster City, CA 94404). Intra-assay CVs are 4.4%, 3.3%, and 4.2% at 14, 41, and 124 ng/ml, respectively. Inter-assay CVs are 6.1%, 6.2%, and 4.7% at 37, 107, and 320 nmol/l, respectively.

25 Hydroxyvitamin D3: using LC-MS (ThermoFisher Scientific, Franklin, Massachusetts 02038 and Applied Biosystems-MDS Sciex, Foster City, CA 94404). Intra-assay CVs are 3.8%, 2.4%, and 4.7% at 25, 54, and 140 ng/ml, respectively. Inter-assay CVs are 6.4%, 6.8%, and 5.0% at 60, 130, and 350 nmol/l, respectively.

1,25 Dihydroxyvitamin D2: using LC-MS (ThermoFisher Scientific, Franklin, Massachusetts 02038 and Applied Biosystems-MDS Sciex, Foster City, CA 94404).). Intra-assay CVs are 7%, 7 %, and 7% at 26, 45, and 307 pg/ml, respectively. Inter-assay CVs are 9.4%, 12%, and 7% at 31, 82, and 287 pg/mL, respectively.

1,25 Dihydroxyvitamin D3: using LC-MS (ThermoFisher Scientific, Franklin, Massachusetts 02038 and Applied Biosystems-MDS Sciex, Foster City, CA 94404). Intra-assay CVs are 5%, 5 %, and 5% at 34, 35, and 301 pg/ml, respectively. Inter-assay CVs are 10.7%, 8.5%, and 8% at 20, 67, and 219 pg/mL, respectively.

The Endocrine Core Laboratory is a participant in the Vitamin D External Quality Assurance Surveillance (DEQAS, program, www.deqas.org) since 2002, and the Clinical Chemistry laboratory partakes in the quality assurance, evaluation, and accreditation from the College of American Pathologists (www.cap.org).

B-Anthropometric measures:

-Neonatal knee to heel length:

Knee-heel length will be measured using hand-held vernier calipers. Knee - heel length measurement is operator dependent; hence measurements will be done in triplicate, and only by pediatricians or neonatologists who are trained on how to use such instruments and who have derived their own precision estimates.

-Neonatal weight: will be done by a trained nurse or research assistant using a regularly calibrated scale.

-Maternal weight: will be done by obstetric staff using a regularly calibrated scale; same scale will be used for all visits.

C-Maternal blood pressure (BP):

It is measured by the obstetric nurse, at each visit, using a regularly calibrated sphygmomanometer. It will be done after that the participant sits and rests for 5 minutes before BP checking. The participant extends her arm and supports it on a flat surface. The arm should be at the same level as the heart.

D-APGAR score:

It is done by pediatric resident at 1 and 5 minutes, as part of routine clinical care.

E-Fetal Ultrasound:

Fetal US is done by the OB-GYN attending as part of the routine prenatal care. In the first trimester, the crown-rump length is measured. In the second trimester, at week 20, the following measurements are taken: head circumference, bipariatal diameter, abdominal circumference, femur length. Fetal US machine is Philips iU22 xMATRIX ultrasound system. Results will be reported in the participant file when she presents for the second study visit.

F- Bone mineral Density:

Neonatal DXA assessment using Hologic machine, Horizon A, version 13.5.3.1, at AUBMC to check bone and fat mass. Whole body scan will be measured. Infant DXA assessment is performed by certified technicians. The technician positions the laser light so that it is centered about 2 cm below the iliac crest (or umbilicus/belly button) on the child, and observes the emerging image to ensure that the spine is centrally positioned and straight, and that the top of the iliac crests and all of L5 are visible. The total radiation dose is 0.04 mSv.

G- Data collection and recording:

Data collection on the baseline participant characteristics will be collected in the initial visit through an interviewer-led questionnaire. Information related to variables that might change during the study (e.g. medications, dietary habits..) will be collected during each visit. Case report forms for data collection will be identical to the forms that are filled online, in order to minimize error.

Adverse Events Forms:

Information related to possible adverse events will be collected at each visit. In addition, pregnant women will be called every 2 weeks and asked about any adverse events.

Retention:
Retention of participants will be improved with the following [5-6]:

- At the participants level:

-Short follow up period: latest follow up is done at 1 month after delivery, not beyond this period in order to avoid non retention.
-Keep contact information for participants up to date.
-Limit the burden and inconvenience of data collection on the participants: avoid redundancy, repetition and meaningless questions, questionnaires used are validated.
-Attendance at follow-up: reminder to each visit (by phone call).
-Keep a good relationship with clinic, decrease waiting time and transportation; Encourage study personnel to show empathy toward subject's personal situation in scheduling appointments and cancellations.
-At delivery, and before discharge the research assistant will remind the participant of out-patient follow-up plan (infant BMD after 1 month)
-Free sessions about breast feeding and newborn care for participants are provided in the women's Health Center at AUB-MC.
-Compensation (transportation fee) for women coming from the collaborating centers (RHUH and Bahman Hospital) to do BMD at AUB-MC
-A picture of the mother and her newborn will be taken by a professional photographer and will be given to the mother.

- At the study staff level:

-Training of the study staff that keeping participants in the trial until the end is crucial. Indeed, they should convey this information to study participants.
-Training of the study staff to be complete in data collection and entry.
All the outcomes will be assessed in participants who discontinue or deviate from the intervention protocol.
Regardless of adherence, all randomized participants will be included in an intention-to-treat analysis.

19-Data management:
-Data entry:
Laboratory values and BMD results will be entered electronically via migration from Hospital Information Systems (HIS) into electronic database specifically created for the study. A manual checking will be done on a random sample of individuals chosen periodically (20% of total number).
All other values will be entered manually. Double entry of data (independently by endocrinology and OB-GYN research assistants) will be done to avoid mistakes. It will be done regularly, following participants' visits. For questionnaires related data, the data entry screens will resemble the paper forms (same order of questions).
Verification of data will be done with range checks, ascending/ descending order view of variables and descriptive histograms that show outliers.

-Coding and terminology:
Non-numeric data are coded using standard coding practices.
Standardized terminology and abbreviations will be used to avoid misinterpretation.
A list including all codes and abbreviations used will be available to individuals responsible of data entry.
Access to data and data entry will require user identification code and password.

-Back-up of data:
A complete back-up of data will be performed twice weekly.

-Meetings:
Trial monitoring committee and all study staff will meet on a weekly basis to review routine operations, trouble shoot, and solve any problem occurring in patient recruitment, data collection and management and make sure that all study steps are implemented as per guidance in the standard operating procedures (SOP).

-Participants files storage:
Participants' files will be stored in numerical order in a locked cabinet, with restricted access (only to study staff). Participant files will be maintained in storage for a period of 5 years after completion of the study.

20-Statistical methods:
See main manuscript

Methods: Monitoring

21-a: Data monitoring—formal committee:
-Data Safety Monitoring Board (DSMB)
See details on page 5.

21-b: Data monitoring—interim analysis
Interim analysis will not be done; previous studies on similar, even higher doses of vitamin D supplementation during pregnancy [1-2] did not result in any case reports of vitamin D toxicity. On the other hand, no early beneficial effects leading to early termination of the study are expected.

22-Harms:
Risk of harm will be assessed by close follow up on adverse events which are defined as follows:
-Adverse Event (AE)
Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

- Serious Adverse Event (SAE):
Any untoward medical occurrence that, at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- results in a congenital anomaly/birth defect

- Adverse event related to interventional drug, is identified as such: when there is a reasonable possibility, in the opinion of the principal investigator, that the experience was more likely than not to have been caused by the research procedures.

-Collection:
Adverse events will be collected regularly:

- After starting the trial intervention drug, during each trial visit, adverse event will be assessed and collected.
- Between visits, the participant will be called by the research team as a reminder for compliance to trial medication; the participant will be asked also about adverse event. If any, information would be obtained, documented in CRF, discussed with TMC, and the participant may be invited for further workup.

-Causality of the adverse event with interventional drug will be assessed as [7]:

Not related
Unlikely to be related
Possibly related
Probably related
Definitely related

-Intensity of the adverse event will be assessed as [8]:

-Mild
-Moderate
-Severe
-Life threatening
-Death

-Expectedness of the adverse event will be assessed as:

- Unanticipated adverse event - any unforeseen or unexpected adverse event which is not described in the general investigational plan, or elsewhere in the current application or with the current investigator brochure, or in the consent document.
- Anticipated adverse event – any foreseen or expected incident which is described in the general investigational plan or elsewhere in the current application or with the current investigator brochure, or in the consent document

We don’t anticipate adverse events in our study since previous trials on vitamin D replacement during pregnancy, using higher doses than ours, did not reveal any adverse events.

Reporting timeframe:
The TMC will report any SAE to IRB and DSMB within 48 hours.

23: Quality control and audit:

Quality control will be insured by the following:

-Before starting recruitment:

-Developing SOPs for all trial events and measurements (Blood pressure, height, weight, BMD measurement ...)

-Using calibrated and standardized instruments (e.g. use of knemometer for knee-heel length measurement)

-Training the research team on all SOPs and ensure their CITI certification and recertification as required by institutional policy.

-Using CRFs that are consistently labeled (patient ID, date, visit number..) that are similar in content and format to the electronic forms, in order to minimize error.

-Selecting computer software (RedCap) for database management that are user friendly, allow alerts in case of missing data, out-of-range values.

-Using electronic forms that are clear, easy to read, coherent and consistent, same layout and format of hard copy forms.

-After starting recruitment:

-Weekly staff meetings of TMC members

-Periodic audit and performance review of 10 % of CRF at each time point (visit). Audits will be performed by independent internal body, designated by TMC in coordination with compliance unit of IRB, if available or by a quality assurance officer working at AUB-MC, hired to perform this function by the TMC, and paid to do so on an hourly basis.

If serious violations are found, re-training of concerned RA will be implemented and a more extensive audit will be performed depending on the situation, in order to introduce appropriate corrective action.

Ethics and dissemination

24-Research ethics approval:
See main protocol manuscript.

25-Protocol amendments:
Any modifications to the protocol (changes to the eligibility criteria, study design, sample size, study procedures, outcomes, analyses) or significant administrative changes which may impact the conduct of the study, potential benefit of the patient or may affect patient safety will be sent to IRB as a formal amendment, prior to implementation. Latest protocol version (number and date) will be used in the study and copies of updated CRFs included with subjects' CRFs. A list of amendments with (corresponding

dates and changes that were done) will be kept with the principal investigator to track the history of amendments.

26a-Consent:

The patient's physician will introduce the study to pregnant women, when they come for their regular prenatal visit (second visit of the first trimester). If she agrees, a trained research assistant will use lay language to explain the trial protocol, make sure that the potential participant understands the research purposes, allow her to ask questions and confirm that her participation is completely voluntary, that withdrawal is possible and will not affect her care at AUB-MC. In light of the information provided, women willing to participate will sign an informed consent, or she alternatively takes it home and get back to the research team the next day. A copy of the informed consent will be given to each participant. (See Appendix 5)

26b-Consent—ancillary studies:

Additional maternal blood and cord blood samples will be collected for genetic studies. Participant's approval for genetic studies will be documented in a separate consent form, related to genetic studies only. They will also be informed that their withdrawal is possible and will not affect their care at AUB-MC. They will be given the opportunity to decide on whether they would like to know or not the genetic studies results.

27-Confidentiality:

All participants' information, local databases and informed consent will be stored in locked cabinets in areas with limited access, and password protected access system. The participants' samples will be de-identified and given coded ID number to maintain confidentiality. One master sheet will be kept under lock and will contain all information regarding identifiers, patient contact information, etc...

28-Declaration of interests:

The investigators have no conflicts of interest to declare.

29-Access to data:

All investigators will be given access to full trial dataset. These data will not contain participants' identifiers but only coded information. Access to data will be password protected.

30-Ancillary and post-trial care:

We do not expect significant adverse events from participating in this study. In fact, trials using similar or even higher doses of vitamin D during pregnancy did not report serious adverse events [1-2]. There will be no compensation to cover expenses related to any adverse event that results from the study and is not covered by a third party or governmental insurance.

Post-trial care: Our study population is pregnant women; by the time the results are out, pregnant women would have delivered. Study subjects will be given the opportunity to receive information on study findings once analyses are completed. However, the findings of our study may guide vitamin D replacement regimen in participants' subsequent pregnancies and in other pregnant women. The wide

availability of the inexpensive vitamin D supplementation would not be a problem to consider post-trial care.

31a: Dissemination policy—trial results:
Trial results will be communicated to participants, to the public and to health care professionals at AUB-MC and in Lebanon. Results will be published in a peer-reviewed medical journal whether the results are in the expected direction or not.

31b: Dissemination policy—authorship:
Individuals who fulfill authorship criteria - those who have substantive contributions to the design, conduct, interpretation, and reporting of the trial – are those who would be trial publication authors (steering committee, TMC and research assistants).

31c-Dissemination policy—reproducible research:
No later than 5 years after the completion of analyses on primary and secondary outcomes we will deliver a completely de-identified data set to an appropriate data archive for sharing purposes.

32-Biological specimens:
Maternal and venous cord blood and placental samples (the latter samples being only form AUB-MC) collected as part of the study, will be preserved de-identified. These samples will be kept up to 10 years after study completion.

References:

1-Hollis BW, Johnson D, Hulsey TC, et al. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res* 2011;26(10):2341-57.

2-Dawodu A, Saadi HF, Bekdache G, et al. Randomized controlled trial (RCT) of vitamin D supplementation in pregnancy in a population with endemic vitamin D deficiency. *J Clin Endocrinol Metab* 2013; 98(6):2337-46.

3-Jones G .Pharmacokinetics of vitamin D toxicity. *Am J Clin Nutr* 2008;88(2):582S-586S.

4- El-Hajj Fuleihan G, Baddoura R, Halaby G, Habib R, Arabi Asma, Rahme M, Singh R, Kassem M, Mahfoud Z, Hoteit M, Daher R, Kassir M. A randomized trial investigating the impact of vitamin D replacement on indices of insulin resistance in elderly overweight subjects. Abstract 1091 Annual meeting of the American Society of Bone and Mineral Research October 9-12, 2015, Seattle, Washington USA.

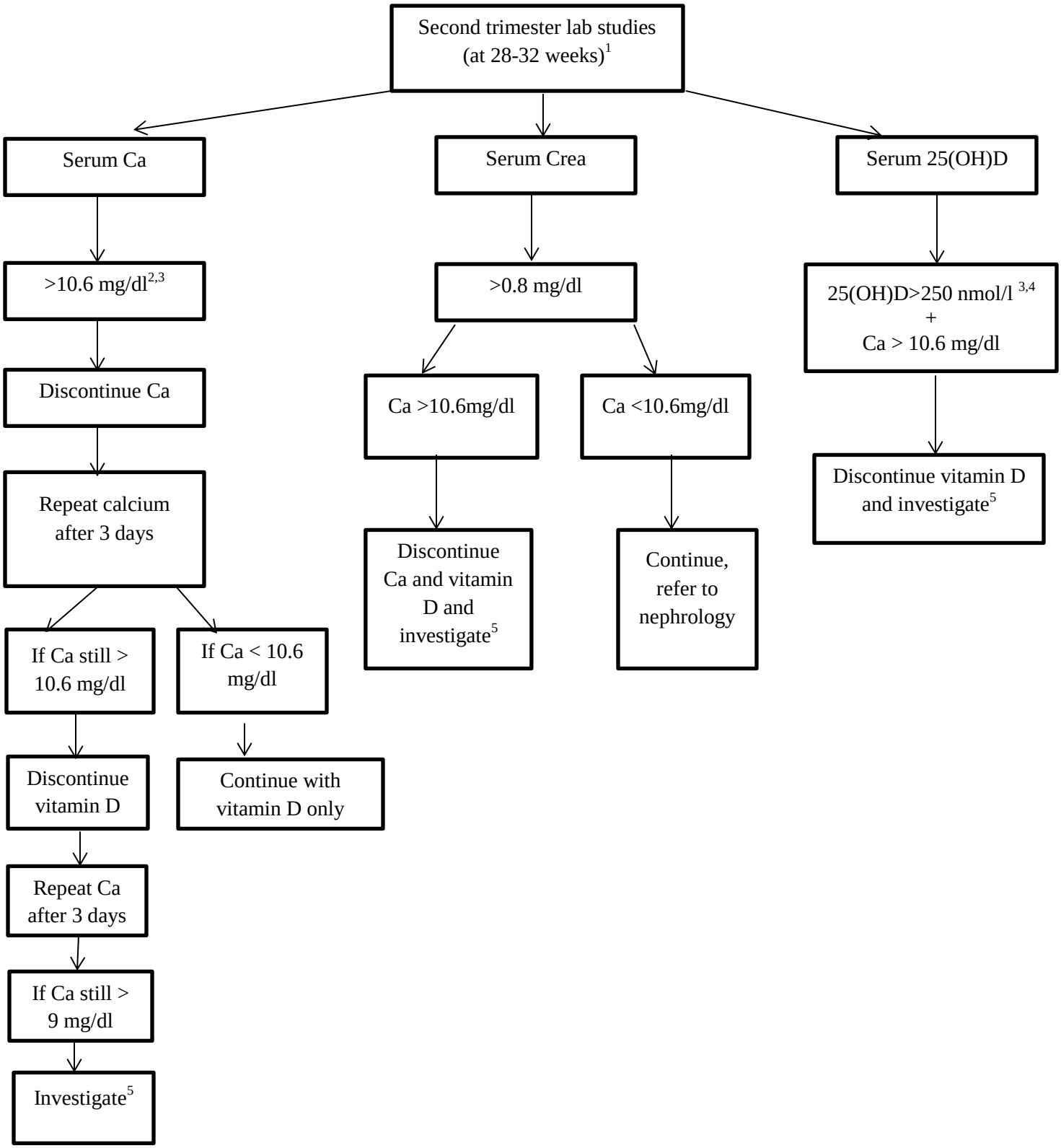
5- Little RJ, D'Agostino R, Cohen ML, et al. The prevention and treatment of missing data in clinical trials. *N Engl J Med* 2012;367(14):1355-60.

6- Robinson KA, Dennison CR, Wayman DM, et al. Systematic review identifies number of strategies important for retaining study participants. *J Clin Epidemiol* 2007;60(8):757-65.

7- NIH Case Report Forms Templates
https://nccih.nih.gov/grants/toolbox

8- NIH Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Appendix 4: Algorithm for the management of hypercalcemia and vitamin D toxicity in the trial.



[1] Urine calcium was not included in the algorithm because of the wide variability in reference range values published in previous studies.
[2] Calcium reference range during pregnancy (mg/dl): 8.2-10.6
[3] Previous studies in pregnant using higher doses of vitamin D supplementation (4000IU daily) did not report any case of hypercalcemia or vitamin D toxicity (Data from Hollis et al. *J Bone Miner Res. 2011;26(10): 2341–2357* and Dawodu et al. *J Clin Endocrinol Metab 2013;98:2337–2346*).
[4] 25(OH)D reference range (ng/ml):

Deficient	< 10
Insufficient	10-25
Desirable	>25
Potentially toxic	>150

[5] Investigation in case of hypercalcemia (with or without vitamin D toxicity) includes:

-Ionized Calcium
-Phosphorus level
-Magnesium
-Albumin level
-1,25$(OH)_2$D
-PTH
-Check medications

Appendix 5: Informed Consent form

Consent to participate in a Research Study

Effect of Vitamin D Replacement on Maternal and Neonatal Outcomes: A randomized Controlled Trial in Pregnant women with Hypovitaminosis D.

Investigator: Dr. GHADA EL-HAJJ FULEIHAN
ADDRESS: AMERICAN UNIVERSITY OF BEIRUT MEDICAL CENTER.
Cairo Street
Beirut, Lebanon
Phone: (01) 737 868 or (01)-350 000 ext 5365

Sites where the study will be conducted:

American University of Beirut – Medical Center (AUBMC), Department of Obstetrics and Gynecology and Department of Endocrinology.
Rafic Hariri University Hospital (RHUH), Department of Obstetrics and Gynecology.
Bahman Hospital, Department of Obstetrics and Gynecology.

You are being asked to participate in a clinical research study conducted at the American University of Beirut Medical Center, Bahman Hospital and at Rafic Hariri University Hospital. Please take your time to read the following information carefully before you decide whether you want to take part in this study or not.
Feel free to ask your doctor if you need more information or clarification about what is stated in this form and the study as a whole.

Why is this study being done?

Vitamin D deficiency is prevalent worldwide and particularly in the Middle East. During pregnancy, vitamin D deficiency may be associated with maternal complications including infections and other metabolic problems such as hypertension and diabetes. In neonates and on the long term run, low vitamin D level may lead to suboptimal bone and muscle development. However, all these findings are not conclusive. There is a great debate among societies and guidelines regarding vitamin D replacement during pregnancy. The WHO guidelines 2012 recommend vitamin D supplementation to pregnant women in populations with a high prevalence of vitamin D deficiency, as is the case in our region. Some scientific societies recommend screening for vitamin D deficiency during pregnancy. The recommended Vitamin D replacement doses vary from 600 to 2,000IU daily. These recommendations target Western populations who tend to have higher vitamin D level in blood. Higher doses are needed in our region. The tolerable upper intake varies from 4,000 IU to 10,000 IU daily.

The purpose of this study is to find the adequate dose of vitamin D supplementation that allows achieving a sufficient maternal blood vitamin D level and ultimately would lead to better long bone health in newborns.

This study will be conducted at AUBMC, in the Women's Health Center private clinics, Obstetrics and Gynecology outpatient clinics (OPD), RHUH, in the Department of Obstetrics and Gynecology and Bahman Hospital in the Department of Obstetrics and Gynecology. The written consent will be restricted to these units only. Up to 280 women will be recruited in this study.

What will happen if you take part in this study?

During your routine second prenatal visit, if you are a candidate for the trial, you will be approached by the research assistant, who will explain to you the objectives of the study and will ask you to participate and sign a consent form approved by the institutional review board. After the informed consent has been signed, the following will be done:

At 11-13 weeks: When you come for the first trimester ultrasound, done as part of the routine prenatal care, blood and urine tests will be performed. Your vitamin D level will be checked. If the level is very low, you will not be eligible to take part in the trial and the research team will arrange for you to receive vitamin D supplementation through your physician. If the level is sufficiently high, you will also not be eligible to take part in the trial.

At 15-18 weeks: If your vitamin D level is intermediate, when you come for your regular prenatal visit at 15-18 weeks, you will meet the research team as a first visit in the trial. During this 30-minute visit, a research assistant will ask you questions about your diet and lifestyle and take some body measurements. You will be given the study medication for the duration of the trial which extends over the whole remaining part of your pregnancy. You will be randomized to receive vitamin D weekly supplementation, EuroD preparation provided by Europharm: 2 tablets of 10,000 IU weekly or 1 tablet of 10,000 IU weekly and 1 tablet of placebo alternating with 2 tablets of placebo weekly.

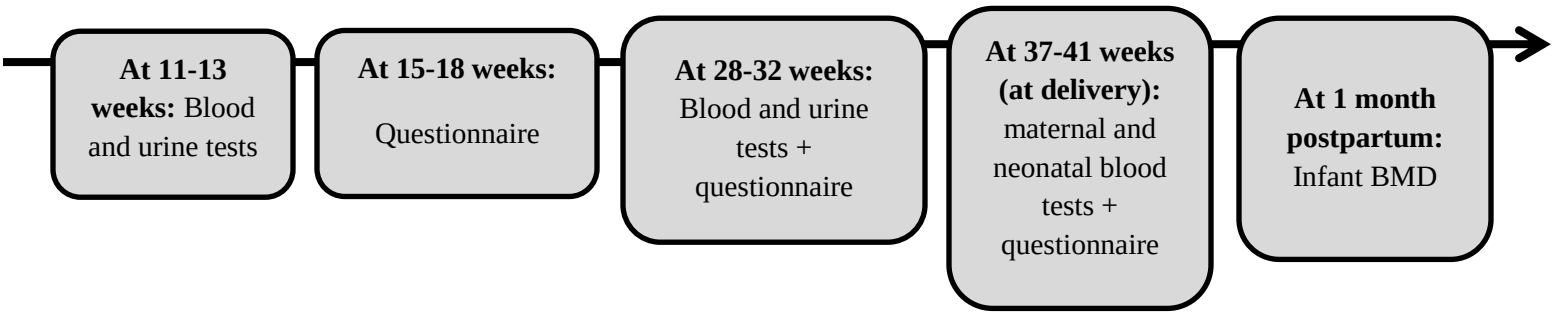
If you present after 13 weeks of gestation, you are still eligible for blood tests given that blood withdrawal occurs before 17.5 weeks and your first visit in the trial occurs before 18 weeks GA.

At 28-32 weeks: When you come for your regular prenatal visit at 28-32 weeks, you will have your second trial 30-minute visit whereby research assistant will ask you questions about your diet , study medication and take some body measurement. A second set of blood will be withdrawn and you will be given instructions to do a 24-hour (or spot) urine collection.

At 37-41 weeks, at delivery : When you come for delivery, you will be visited – a 30-minute visit - by the research assistant who will ask you questions about your diet, study medication and details (maternal and newborn) about the delivery. The research assistant will also measure the height of your spouse. Blood tests will be withdrawn for a third set of tests and samples will be

collected from the afterbirth (blood from the cord) .This will have no effect on the baby whatsoever.

At 1 month postpartum: The research team will arrange for you and your infant an appointment to do Bone Mineral Density (BMD) at 1 month postpartum. During this visit, the research assistant will ask you questions about your infant health and diet and your infant undergoes BMD measurement. This visit duration is 30 minutes.



The research team will notify you of any new significant findings about the study should they surface.

The maternal blood volume withdrawn at each study visit ranges from 6 to 22 ml, depending on the visit, which is equivalent to 0.5-1.5 tablespoons. The total amount of maternal blood withdrawn for the whole study doesn't exceed 44 ml (equivalent to 3 tablespoons) and 7 ml of the venous cord blood (equivalent to 0.5 tablespoon).
The amount of urine required for spot urine studies, when needed, is 5 ml.

How long will I be in the study?

This study extends from the first trimester of pregnancy until one month postpartum. The researcher may decide to take you off the study if he feels that it is in your best interest, if you are not able to follow the rules of the study, if the study is stopped before it is completed, or if new information becomes available that indicates it would be best for you to stop being in the study.

You can choose to stop taking part in this study at any time. If you decide so, you should let the researcher or his staff knows so that they can make sure you are safely taken out of the study.

What are the possible disadvantages and risks in taking part?

There is a remote risk of developing hypercalcemia/toxicity with vitamin D doses of 50,000 IU daily for weeks to months, doses that are 16 folds higher than the doses used in our trial. Hypercalcemia/toxicity manifests with dry mouth, kidney stones, headache, nausea, vomiting,

lethargy, confusion, abdominal pain, arrhythmia. However, doses of vitamin D supplementation of 4,000 IU daily, higher than the doses used in this study, have been shown to be safe in 180 pregnant women, in 2 recent studies. None of these women had any sign of toxicity.

Blood withdrawal is associated with a minimal risk routinely encountered during any peripheral sampling: skin infection, phlebitis, or hematoma. Blood withdrawal will be performed by physicians or trained personal in order to further minimize such risks. The extra blood tests to be done will be ordered free of charge and will not cost you any money.

If your child takes part in this research, he or she will have one medical imaging study which uses radiation. The test or treatment your child will have includes a bone mineral content measurement. The radiation exposure from this research is about 7 microsievert. To give you an idea about how much radiation your child will get, we will make a comparison with an every-day situation. Everyone receives a small amount of unavoidable radiation each year. Some of this radiation comes from space and some from naturally-occurring radioactive forms of water and minerals. This research gives his or her body the equivalent of about 20 extra hours worth of this natural radiation. The radiation dose we have discussed is what your child will receive from this study only, and does not include any exposure he or she may have received or will receive from other tests (http://www.safety.duke.edu/Radsafety/consents/default.asp).

The DXA scan will be performed once, after the infant feeding and while asleep, to minimize motion artifact.

In the rare event of motion artifact, the scan will be aborted and the infant will be excluded from the study.

What are the possible benefits of taking part?

Direct benefits include:

-You will be able to know your vitamin D level. If it is very low, you will receive treatment. If it is intermediate, you will receive vitamin D supplementation to improve vitamin D levels that have been shown associated with improved maternal and neonatal outcomes.

-You will be given a picture of your new born baby to take home.

-You will have a free measurement of your infant bone and fat mass.

Indirect benefits include:

-This study allows a better knowledge about the adequate vitamin D supplementation dose during pregnancy to achieve a sufficient vitamin D level that would be associated with improved skeletal and extra skeletal outcomes, both for the mother and the newborn. These findings will be

published in medical journals, and possibly in the local and national press. You will not be identified in these reports/publications in any way. Ultimately, we hope that this study will inform government policy makers.

You will not be paid for participating in this study.

-If you are a participant from a hospital other than AUB (RHUH or Bahman Hospital), you will be reimbursed for transportation costs for the fourth study visit that will take place at AUB-MC.

What about confidentiality and sharing of samples?

If you agree to participate, the information will be kept confidential. Your name or other personal identifiers will not be used during the study. All information about you from this research project will be kept in a locked office or another locked area. Information that is kept on computers will be kept safe from access by people who should not see it. Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental agencies will have direct access to your medical records which may be audited without violating confidentiality. The results of this study may be published in a scientific book or journal. If this is done, your name will not be used.

Storage of research data will be password-protected. Sharing or disclosing research data to collaborators will be carried out in a coded manner only. Equally, samples will be stored in a coded manner. Storage will take place at AUB-MC facilities in a locked area. Only research personnel have access to your coded samples. Information about the code on samples and their links to your identity will be kept in a secure location and access limited to the principal investigator, Dr. Ghada El-Hajj Fuleihan, and her research team.

Sharing of samples with other investigators/collaborators (inside AUB & outside AUB) will be done in a coded manner.

Please indicate your free will as per the below options regarding sharing samples with other investigators as part of this current project:

□ I permit sharing my coded samples with other investigators /collaborators (inside AUB & outside AUB) for further studies that might arise as part of this project.

□ I do not permit sharing my coded samples with other investigators /collaborators (inside AUB & outside AUB) for further studies that might arise as part of this project.

Also please indicate your free will as per the below options regarding using and sharing samples with other investigators for future research other than the current project:

□ I permit further use and share of my coded samples for future research after contacting me for permission.

□ I permit further use and share of my coded samples for future research without contacting me for permission.

□ I do not permit further use and share of my coded samples for future research.

You have not given up any of your legal rights by signing this form.

Is there a compensation for Adverse Events?

We do not expect significant adverse events from participants in this study. In case of any adverse event as a result of the study, there will be no compensation to cover such expenses in case it is not covered by a third party or governmental insurance.

Sharing of results:

Study subjects will be given opportunity to receive information on study findings once analyses are completed. However, the findings of our study may guide vitamin D replacement regimen in the participants' subsequent pregnancies and in other pregnant women.

Investigator's Statement:

I have reviewed, in detail, the informed consent document for this research study with _____ (name of patient, legal representative, or parent/guardian) the purpose of the study and its risks and benefits. I have answered to all the patient's questions clearly. I will inform the participant in case of any changes to the research study.

_____ _____

Name of Investigator or designee Signature

_____

Date and time

Patient's Participation consent:

I have read and understood clearly all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. Ghada El-Hajj Fuleihan at 01-737868 or 01-350000 ext 5365 or any of her designee involved in the study in case of any questions. If I feel that my questions have not been answered, I can contact the Institutional Review Board for human rights at 01-350000 ext 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time, even after signing this form, and it will not affect my care or benefits.

Based on the above, I voluntarily and personally agree to what is mentioned in this consent form. I have received a copy of this signed informed consent.

Name of Patient _____ Signature _____

Date and time _____

Witness's Name _____ Witness's Signature _____

Date and time _____

Parental consent to do BMD in the infant:

For infant bone mineral density measurement, approval of both parents is required:

Name of Mother _____ Signature _____

Name of father _____ Signature _____

Date and time _____

Patient's approval to be re-contacted for future studies.

□ I accept to be re-contacted for future studies.

□ I refuse to be re-contacted for future studies.