In vivo and Ex-vivo Investigation of the Effect of Ascorbic acid and Cobalamin on Uterine Activity in Mice

Enitome E. Bafor, Ekeleoseye Anthea Okosun, Osemelomen Omoruyi, Uloma B. Elvis-Offiah, Gerald I. Eze, Osamwonyi Igbinuwen, Kadiri P. Braimoh

1Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria
2Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Nigeria
3Department of Anatomy, School of Basic Medical Sciences, University of Benin, Nigeria
4Haematology Department, University of Benin Teaching Hospital, Benin City, Nigeria
5Histopathology Department, University of Benin Teaching Hospital, Benin City, Nigeria

# Presenter of research at UBARD

Extended Abstract (Ongoing Research)

Introduction: Many patients now search for alternatives to conventional medicine for management of health disorder including reproductive disorders (Proctor & Murphy, 2001; Liu et al., 2009; Hegde et al. 2007) and studies have shown that nutritional intake and metabolism may play an important role in the causes and treatment of menstrual disorders (Proctor & Murphy, 2001). Since 1994, vitamins, minerals, herbs, amino acids and other dietary substances have been classified as dietary supplements (Dror and Allen, 2012). The water-soluble vitamins B_6, B_12 and C have been suggested to play important roles in maternal health as well as foetal development and physiology during gestation (Dror and Allen, 2012). A clinical trial study showed that vitamin B_6 (pyridoxine) was more effective at reducing dysmenorrhea than both placebo and a combination of magnesium and vitamin B_6 (Davis 1988). Another trial study reported vitamin B_1 (Thiamine) to be effective in the pain of dysmenorrhea when taken at 100 mg daily (Gokhale 1996). Serum levels of vitamin B_12 (Folate) have been found to be lower in users of the Pill than in nonusers (Shojania & Wylie, 1979; Wilson et al., 2011). Low serum cobalamin concentrations have also been detected in women in the last trimester of pregnancy (Baker et al., 2002). This may infer a role for these vitamins in maternal and foetal health. Vitamin C (Ascorbic acid) at dietary doses (100 mg/d) has been reported to prevent premature rupture of the chorioamniotic membrane in pregnancy by modulating collagen metabolism and favouring its deposit in foetal tissues, including the amniochorion membranes (Casanueva et al., 2005). Oxidative stress plays a role in the aetiology of pre-eclampsia and vitamin C has been suggested to prevent pre-eclampsia (Kiondo et al., 2012). Human fertility is believed to be associated with the stimulatory effect of vitamin C on progesterone (P4) and oestrogen (E2) production (Wu et al. 2008; Luck & Jungclas 1988; Luck 1990). There have however been no studies to investigate the direct effect of these vitamins on
uterine contractility. This study is therefore aimed at investigating the effect of water soluble vitamins on uterine contractility and female reproductive function using mice models.

**Hypothesis:** It is therefore hypothesized that water-soluble vitamins may have direct effects on uterine contractility that makes them useful therapeutic agents in reproductive disorders in which uterine contractility plays a major role.

**Methodology:** Female laboratory mice of the Swiss albino strain (20 - 30 g) were employed in this study. The animals were housed at the Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The animals were housed in standard cages at environmentally controlled room temperature and of adequate ventilation. The animals were also provided with adequate food and water *ad libitum*. Adequate hygiene was maintained daily through regular cleaning and removal of faecal matter and leftover feed from the cages.

**Uterine Contractility Assays:** Only animals in oestrous or metestrus stages were employed. The stage of oestrus was determined from examination of vaginal smears. Vaginal smears were obtained by careful flushing of the vagina with normal saline with the aid of a pipette. On the day of the experiment, the vaginal smears were examined and uterine horns carefully dissected out, freed of adhering mesenteric tissues and fat and transected medially. The uterine segments (measuring between 0.5 – 0.8 cm in length) were mounted in 10 ml organ baths containing warmed (37 °C) aerated physiological saline solution (PSS) of the following composition: 154.0 NaCl, 5.63 KCl, 0.648 CaCl₂.2H₂O, 5.95 NaHCO₃ and 2.77 D-glucose (mM). Tissues were placed under 0.5 g tension and then equilibrated for 30 min. The differential force and frequency of spontaneous contractions in the longitudinal muscle layers of each uterine tissue segment were recorded via a 7003E-isometric force transducer (Ugo Basile, Varise, Italy) connected to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (Ugo Basile, Varese, Italy). The effects ascorbic acid, cobalamin and vitamin Bcomplex on uterine contraction during the last 3-min incubation period were recorded and expressed as a percentage change of the control. Time-matched controls will be performed where appropriate.

**Experimental Protocol for the In Vitro Assay:** The effect of the vitamins were determined using the following protocols:

1. Effect on spontaneous uterine contractions (Lijuan et al. 2011); n = 5 animals;
2. Effect on oxytocin-induced uterine contractions (Bafor et al. 2013); n = 5 animals

3. Effect on high KCl-induced uterine contractions (Lijuan et al. 2011); n = 5 animals

**Effect on the Reproductive Function:** The oestrus cycle stage for each mouse were determined daily via vaginal smear observations (Green 1966; Pritchett & Taft 2007; Elvis-Offiah & Bafor 2014). Staging was concluded after one week and the animals were grouped according to the stage of their oestrus cycle; Group 1 received 0.2 ml of 5% Tween 80 p.o.; Group 2 received 150 mg/kg cobalamin p.o.; Group 3 received progesterone 10 mg/kg s.c.; and Group 4 received oestrogen (1 mg/kg p.o.) Drugs and control were administered till a complete cycle was reached and macroscopic and microscopic observations were performed during treatment periods. At the end of the treatment, the animals were euthanized (80 mg/kg pentobarbital i.p.), cardiac puncture was performed, the ovaries, and cervix were separated from the uterine horns and placed in a dish containing 10 % formalsaline. The uterine horns were weighed and placed in 10 % formalsaline. Subsequently, isolated organs were submitted for histological examination. Blood samples were placed in lithium heparinized bottles and submitted for hormonal and hematological analyses.

**Data Analysis and Presentation:** The data are presented as mean ± SEM, n = the number of animals per experiment. Inferential statistics using one-way ANOVA with Dunnett’s post hoc was performed.

**Results/Discussion**

Ascorbic acid and cobalamin have been shown for the first time to have direct effects on uterine contractility with ascorbic acid showing a greater reduction in the force and frequency of uterine contraction. This therefore proposes for the first time a direct role for water-soluble vitamins on uterine contractility. Data analyses are currently ongoing for other datasets from this study, which will, provide more insight into the mechanism of activity as well as in vivo activity of the water soluble vitamins. This study places the University of Benin at the fore of research into reproductive health both locally and internationally and contributes to the global health goal of seeking new interventions in maternal and foetal health.
Figure 1. Concentration-response curves showing the effect of ascorbic-acid (ASA) and cobalamin (CBL) on spontaneous uterine contractions. ASA was observed to decrease the amplitude and frequency of spontaneous contractions (A and B above respectively) while CBL was observed to also decrease the amplitude and frequency of contractions (C and D above). n = 4 animals.

References


