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Biochemical analysis of ascorbic acid assay in human saliva throughout menstrual cycle

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ABSTRACT

The recent finding shows that the ascorbate involves in gamete protection and tissue remodelling. The damage of gametes by free radicals prevented by ascorbic acid and on the whole the preliminary exploration of the ascorbic acid involves directly or indirectly in fertilization or its interrelated actions. Twenty-three females, aged between 18 and 40 years, were recruited for the study. Subjects were not using oral contraceptive pills and had regular menstrual cycles. Ascorbic acid level was measured daily in the first morning specimen of saliva in five ovulating and non-ovulating females who were receiving 500 mg ascorbic acid each morning at breakfast throughout the menstrual cycle. Ascorbic acid level showed massive variations associated with an alteration in basal body temperature and trans-vaginal sonograph (≤ 4 mm size) during the 14th day of the ovulation phase. There was a slight fluctuation during the subsequent fall in ascorbic acid secretion on day 16, and the flow of ascorbic acid diminished further until the 18th and 19th day. The pattern of ascorbic acid secretion closely resembles the flow of luteinizing hormone (LH) at the time of human ovulation. These results provide direct evidence on which theories for the women ovulation cycle can be assessed.

Keywords: Ascorbic acid; Saliva; Menstrual cycle; ovulation

INTRODUCTION

The role of reactive oxygen species and free radicals are involved in the pathogenesis of several medical conditions associated with aging, cancer and fertility well documented and may have evolutionary implication,[1] but its precise physiological role in reproduction has been uncertain. Recent data suggest that ascorbate have defined functions in hormone secretion, gamete protection and gonadal tissue remodelling. Its effect can be explained by cellular and biochemical mechanisms similar to those applicable in other tissues. [1, 2] Vitamin C helps by starving the uterus of progesterone, which then helps in breaking down the uterus walls triggering the menstruation. Ascorbic acid has been associated with fertility, but no consistent study of its mechanism of action in reproductive tissues has been made. Gonads exhibit cycles of tissue remodelling peptide and steroid secretion which can be assumed as ascorbate-dependent. Ascorbic acid may also prevent gametes from damage by free radicals during production and fertilization. Preliminary data on the concentration of ascorbic acid in saliva from women undergoing *in vitro* fertilization vary during the ovulation phase, particularly due to LH dependency. [2, 3] The supply of ascorbic acid to the ovary might limit the ability of the pre ovulation follicle to grow in response to the gonadotropin stimulation. [3] There are divergent views about whether the difference in ascorbic level is due to increased demand for vitamin C or hormonal changes or decreased dietary intake [3-5] According to Nino and Shaw, [6] decreased maternal ascorbic acid level is associated with prematurely birth, pre-enclampsia, anaemia and premature rupture of the foetal membrane. Nelson and Tagfer [7] suggested that the deficiency of ascorbic acid have a teratogenic effect. Ascorbic acid levels were found to be significantly higher in the follicles of women who took vitamin C supplements when compared to

the control group. Vitamin C supplementation was also found to have a direct impact on the number of successful pregnancies. The present study examines the variations in salivary ascorbic acid and steroid hormones as LH and FSH during the menstrual cycle.

MATERIALS AND METHODS

Collection of samples

All the participants gave written informed consent to participate in the study; the protocol designed was approved by Institutional Review Board. The saliva of women was collected as per the spitting method [28] and the collection period being about 10 min. The specimen was processed in pre weighed ice-chilled tubes. Twenty-three females, aged between 18 and 40 years, were recruited for the study. Subjects were not using oral contraceptive pills and had regular menstrual cycles. Food and beverage intake was recorded to avoid food excessive addition of ascorbic acid, using the estimated daily record method for two days during each menstrual cycle (pre ovulation phase (6-12 days); ovulation phase (13-14 days); post ovulation phase (16-26 days) using physical changes like fern examination and temperature chart are used to detect the day of LH surge. During the menstrual cycle, the probing depth ≥4 mm of follicle size during the pre ovulation phase was also assessed through trans-vaginal sonograph to validate the day of ovulation.

Ascorbic acid assay

Samples treated with 3% (w/v) meta-phosphoric acid were stored overnight at 4°C and centrifuged at 4000 rpm for 10 min to remove precipitated protein. Ascorbic acid was assayed colorimetric assay. Plasma ascorbic acid concentration was determined using spectrophotometer using 2, 4-dinitrophenylhydrazine. [15] Ascorbic acid is represented in mg/dL as well as in µmol/L for comparison with earlier data.

Hormone assay

Salivary hormone assay comprising LH and FSH was analyzed in all three phases of the menstrual cycle to detect the exact time of ovulation. The intra-assay and inter-assay coefficients of variation were computed following Diametra, Italy and followed by Salimatrics salivary hormone analysis kit model system was used for interpretation of data. The sensitivities of luteinizing hormone (LH) were 14 mIU/L and serum correlations were 0.80. The intra-assay variations for LH were 4.5 mIU/L. All samples were run in one assay to avoid inter-assay variation.

RESULTS AND DISCUSSION

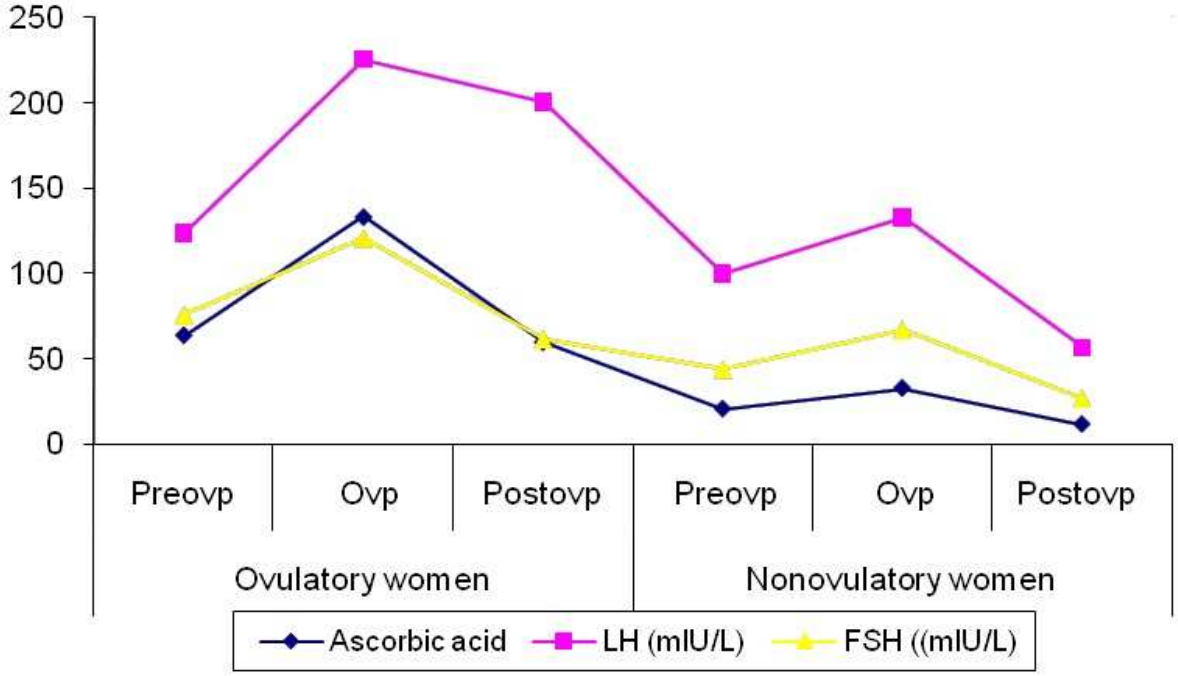
Figure 1 shows the mean ascorbic levels of reproductive phases of women. The ascorbic acid level at delivery was significantly (P<0.05) higher in the saliva in the ovulation phase than in other phases. However, no significant difference between LH and FSH during the pre ovulation and post-ovulation phases was observed in non ovulation subjects. The mean and maximum probing depth is highly significant in the fertile women i.e. ovulation phase show 85% of salivary ascorbic acid in relation to hormonal reliability (P<0.05).

Changes in ovarian content occur at ovulation in guinea pigs and rats; [8] in cows the bovine follicular fluid level is higher during the early (days 1-10 after ovulation) part of the cycle. [9, 10] Women receiving steroidal contraceptives have reduced levels of ascorbic acid in plasma and leukocytes. [11-14] A change in the retention and excretion of ascorbic acid occurs at mid-cycle in women, associated with LH secretion and temperature rise, which has been proposed as a definitive marker of ovulation. [15, 16] There appears a biphasic change such that the excretion increases in the late follicular phase, declines immediately prior to ovulation, and again increases after the rise in body temperature. [17] The immediate cause of these changes is not clear, but the authors have assumed that they reflect changes in the uptake of ascorbic acid by the pre ovulatory ovary. It has been suggested that the changes in retention before ovulation facilitate the luteal steroidogenesis [18] that also explains its cycle-protective effects [19]. More recent studies with luteinizing granulosa cells show that ascorbate stimulates to progesterone and oxytocin secretion. [20, 21] this is consistent with its known roles in hormone biosynthesis, and synergizes with neurotransmitters in stimulating hormone secretion. [22] Notwithstanding these effects, the concentration of ascorbic acid in the corpus luteum appears to be more than the required amount to facilitate hormone production. [23, 24]

Vitamin C has been suggested as a regulator of female fertility. Ascorbic acid supplementation enhanced the ovulation-inducing effects of clomiphene by an apparently confined ovarian effect, [25] and an early study reported that in the infertile cow is prevented conception in otherwise infertile cows after administration of ascorbic acid at estrus. [9] In contrast, the contraceptive action of high doses of ascorbate has also been proposed, based on alterations in the structure of cervical mucus. [13] Large quantities of ascorbic acid are utilized during human conception that is necessary to maintain the integrity of the fetal membranes. [26] Dietary supplementation dose of ascorbic acid during pregnancy may reduce the frequency of birth defects; [7] a daily supplement of at least 500 mg of vitamin C, starting

from early pregnancy has been suggested. [27] However, none of these proposals has been rigorously examined or translated into clinical practice. In present study intake of food and beverages were not contain ascorbic acid to maintain the concentration of ascorbic acid.

Figure 1: Shows the salivary Ascorbic acid, LH and FSH levels of reproductive phases of women. Preovp - Preovulatory period, Ovp - Ovulatory Peroid, Postovp - Postovulatory period



CONCLUSION

Several physiological and biochemical mechanisms, particularly in specific cells and tissues, remain to be investigated, but there is sufficient evidence to indicate the importance of ascorbic acid in the reproductive process. Domestic and other mammals are capable of synthesizing ascorbate, and meet the reproductive demand will be met endogenously, although sub-fertility may result from under-production. In the human, the nutritional obligation means that the level of intake and the metabolic requirement (both alterable by lifestyle) become the major variables in the multi-factorial equations of fertility. The available human data suggest that a re-examination of the clinical potential of vitamin C be timely. A complete understanding of the ascorbate dependence of reproduction as regulations of human reproduction is of immediate practical interest.

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