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MINIREVIEW
Ascorbic Acid and Fertility[1]

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ABSTRACT

Ascorbic acid has long been associated with fertility, but no consistent study of its mechanism of action in reproductive tissues has been made. This article considers how three of ascorbic acid's principal functions, namely its promotion of collagen synthesis, its role in hormone production, and its ability to protect cells from free radicals, may explain its reproductive actions. Data relating to both ovary and testis are reviewed since ascorbate accumulates in both tissues. Both gonads exhibit cycles of tissue remodeling and of peptide and steroid secretion that can be assumed to be ascorbate-dependent. Ascorbic acid may also prevent gametes from damage by free radicals during production and fertilization. Preliminary data on the concentrations of ascorbic acid in serum and follicular fluid from women undergoing in vitro fertilization are presented. They suggest that the supply of ascorbic acid to the ovary might be a limiting factor in the ability of the preovulatory follicle to grow in response to gonadotropin stimulation. It is concluded that ascorbic acid is a key compound in gonadal physiology on which further research is needed and that a reappraisal of its potential clinical value in the treatment of various types of male and female infertility would be timely.

INTRODUCTION

Ascorbic acid is an essential component in the diet of humans and a small range of other mammals. It has been associated with fertility for many years and may have evolutionary significance [1], but its precise physiological role in reproduction has been uncertain. Recent data suggest that ascorbate has defined functions in hormone secretion, gamete protection, and gonadal tissue remodeling. Its effects can be therefore be explained by cellular and biochemical mechanisms similar to those applicable in other tissues [2, 3]. This review suggests that ascorbate should be considered as an essential biochemical in the reproductive process and as a potentially significant factor in human fertility.

BIOLOGY AND PHYSIOLOGY OF ASCORBIC ACID

L-Ascorbic acid is a six-carbon keto-lactone (Fig. 1), M_r 176.13, synthesized from glucose via several intermediates [2, 4]. As a result of an ancient gene mutation [4], primates, guinea-pigs, and bats [5] lack one of the liver enzymes in its biosynthesis, L-gulono-lactone oxidase. They therefore require a dietary supply of the vitamin. For primates and guinea-pigs, the primary sources (fruit and vegetables) are well known. Not all the vitamin-dependent bats are obligate herbivores, and their dietary source is uncertain [6]. The human requirement, expressed as a recommended daily intake, has been much debated and varies with lifestyle and physiological status [2, 5, 7].

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A range of body tissues accumulate the native vitamin and its fully active, oxidized form, dehydroascorbic acid. Tissues vary widely in content but the highest concentrations occur in the pituitary, adrenal gland, and gonads [2, 8–11]. Measurements of ascorbate in serum or plasma [8, 12, 13] show no consistent difference between the sexes, but it is unlikely that these estimates accurately represent the circulating level. Leukocytes, particularly neutrophils, possess very high concentrations [14] and are probably the primary vehicles for its distribution around the body. The average half-life in humans is about two weeks, with a turnover of 1 mg/kg/day and a body pool of 22 mg/kg [15].

Ascorbic acid has three biological actions of particular relevance to reproduction, each dependent on its role as a reducing agent: it is required for the biosynthesis of collagen, for the biosynthesis of steroid and peptide hormones, and to prevent or reduce the oxidation of biomolecules. It is frequently involved in mixed-function oxidation, resulting in the incorporation of oxygen from molecular oxygen into a substrate [16].

The role of ascorbic acid in maintaining the structure of collagenous tissues is well known for historical reasons [2]. It is needed for the synthesis of collagen during tissue development and at sites of tissue damage, and also for the maintenance of the slow collagen turnover which occurs in mature tissues [17]. Acting as an electron donor, it is an essential co-factor for the enzymes that hydroxylate proline and lysine residues during the post-translational processing of pro-collagen [17, 18]. Hydroxylation occurs on residues located on the amino sides of regularly occurring glycine residues in the collagen precursor, and permits cross-linking and glycosylation. In the absence of ascorbate, the procollagen is unable to assume its trihelical conformation and cannot be secreted by the cell [17, 18]. Ascorbate is also

reported to promote collagen production at the gene level [19].

The high concentrations of ascorbate in endocrine tissues attest to its importance in hormone synthesis. The neurohypophysial synthesis of oxytocin and vasopressin involves a post-translational amidation that is ascorbate-dependent [20], and neurotransmitter synthesis in other areas of the brain is similarly promoted [21, 22]. Catecholamines require ascorbate for their synthesis and to protect them from oxidation [23, 24]. Steroidogenesis appears to be ascorbate-dependent [25], particularly at hydroxylation steps [26].

The antioxidant properties of ascorbic acid enable it to protect tissues from reactive oxygen species such as $O2^-$, OH^-, H2O2, $^1O2$, OCl^-, NO, and metal-oxygen complexes [27–29]. These radicals can be damaging to DNA, proteins, carbohydrates, lipids, and biological membranes, sometimes with pathological consequences.

ASCORBIC ACID AND THE TESTIS

Early studies reported direct effects of ascorbate deficiency on male fertility in laboratory and farm species [30–32]. Low levels of ascorbate in bull semen were associated with poor breeding performance, while scorbutic guinea pigs experienced degeneration of the testicular germinal epithelium. In rabbits, the gonadal growth-enhancing effects of gonadotropins could be significantly enhanced by simultaneous treatment with ascorbic acid. These studies suggest that ascorbate affects both the integrity of the tubular structure and functionality of sperm. In the case of the tubule, it can be assumed that ascorbate is required for secretion and maintenance of the layers of collagen types IV and I, which form a major part of the complex basal lamina [33], although direct experimental evidence of this is lacking. At the endocrine level, ascorbate stimulates the secretion of an oxytocin-like peptide by guinea-pig Leydig cells [34], presumably by facilitation of peptide amidation.

Low or deficient ascorbate levels have been associated with low sperm counts, increased numbers of abnormal sperm, reduced motility, and agglutination [35–38]. In some of these studies, dietary treatment was found to improve sperm quality. The beneficial effects of ascorbate on sperm may result from the destruction of free radicals, present as a consequence of environmental pollution and cellular metabolism, which would otherwise cause oxidative damage to DNA [38]. It has been suggested that a genetically defective sperm population might result in heritable mutations, genetic birth defects, and cancer, particularly in populations at high risk of oxidative damage such as smokers [39]. Dawson et al. [38] concluded that male fertility in general would be improved by an increased dietary vitamin C intake.

ASCORBIC ACID AND THE OVARY

The ovary has long been recognized as a site of ascorbic acid accumulation and turnover, with the highest concentrations in the theca interna, granulosa, and luteal compartments [40, 41]. The ability of LH to block the uptake of ascorbic acid by gonadotropin-primed rat ovaries [42] provided the basis for a bioassay [43, 44] that was much exploited before the advent of immunoassays. Changes in ovarian content occur at ovulation in guinea pigs [45], rats [46], and cows [47], and the level in bovine follicular fluid is generally higher during the early (Days 1–10 after ovulation) part of the cycle [48]. Women receiving steroidal contraceptives have reduced levels of ascorbic acid in plasma and leukocytes [8, 49–51].

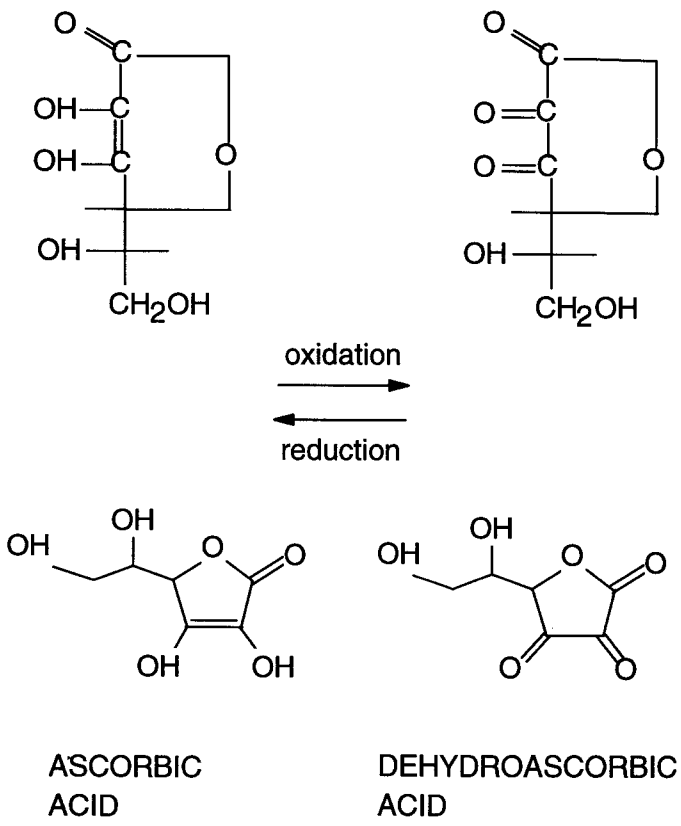

FIG. 1. Alternative depictions of the structure of L-ascorbic acid and its biologically active oxidation product, dehydroascorbic acid.

A change in the retention and excretion of ascorbic acid occurs at mid-cycle in women, associated with LH secretion and temperature rise, and has been proposed as a definitive marker of ovulation [52, 53]. There appears to be a biphasic change such that excretion increases in the late follicular phase, declines immediately prior to ovulation, and increases again immediately after the rise in body temperature [54]. The immediate cause of these changes is not apparent, but authors have assumed that they reflect changes in the uptake of ascorbic acid by the periovulatory ovary. It has been suggested that changes in retention before ovulation facilitate luteal steroidogenesis [55], and that this relationship also explains its cycle-protective effects [56]. More recent studies with luteinizing granulosa cells show that ascorbate is stimulatory to progesterone and oxytocin se-

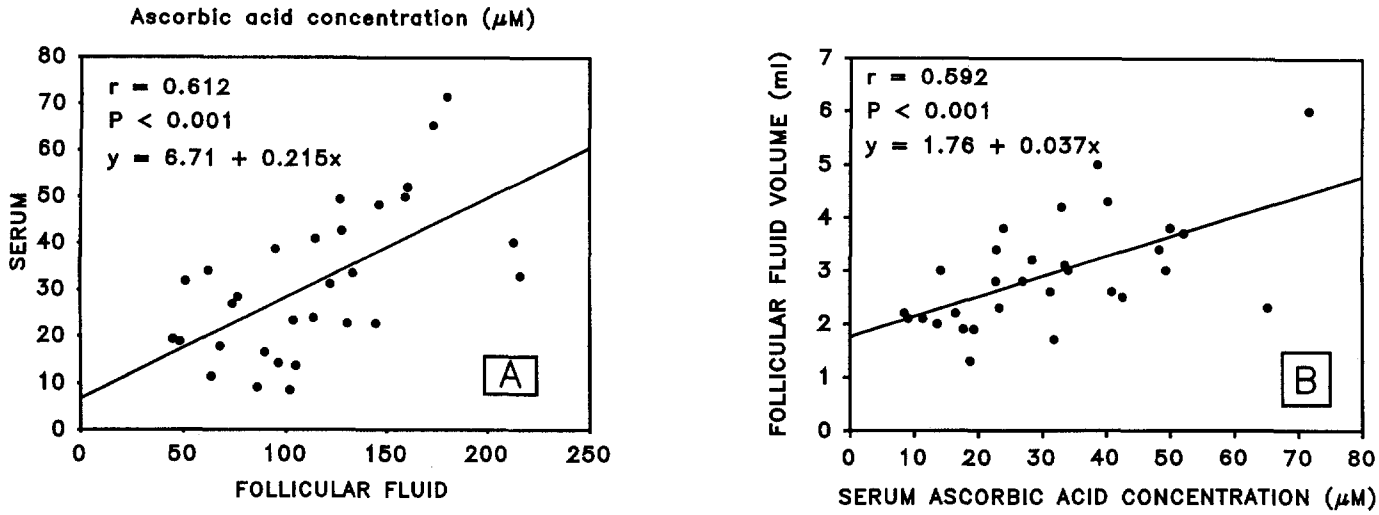

FIG. 2. Regression analyses of relationships (A) between ascorbic acid concentrations in serum and follicular fluid, and (B) between follicular fluid volume and serum ascorbic acid concentrations, in samples taken from 30 women undergoing IVF procedures. Fluid was obtained from the largest follicle punctured at the time of oocyte collection following stimulation with pergonal and hCG. Serum was obtained at the same time. Samples were treated with 3% (w/v) metaphosphoric acid, stored overnight at 4°C, and centrifuged to remove precipitated protein. Ascorbic acid was assayed colorimetrically [76].

cretion [57, 58], consistent with its known roles in hormone biosynthesis, and synergizes with neurotransmitters in stimulating hormone secretion [59]. Notwithstanding these effects, the concentration of ascorbic acid in the corpus luteum appears to be greatly in excess of that required to facilitate hormone production [60, 61].

An additional explanation for its abundance in the ovary concerns the high rates of tissue remodeling and collagen synthesis that attend the follicular-luteal cycle [32, 62, 63]. Collagen synthesis is required for follicle growth, for repair of the ovulated follicle [64], and for corpus luteum development [61]. Ascorbate will also be needed for secretion of collagen and proteoglycans into follicular fluid [65–67]. To gauge the requirement during follicle growth, the follicular basement membrane and theca can be considered as the surface of a growing sphere whose quantity will increase as the square of follicular radius. Since the radius may double on a daily basis, the local demand for collagen synthesis, and for ascorbate, will be intense, particularly in species (human, cow) with relatively large mature follicles. These concepts have yet to be investigated directly, but an early study of infertility in scorbutic guinea pigs [68] describes degeneration in the follicle wall consistent with a loss of basement membrane integrity.

In a preliminary study (I. Jeyaseelan and M.R. Luck, unpublished) of ascorbic acid concentrations at the time of oocyte recovery in women undergoing IVF procedures, we observed a strong correlation between follicular fluid and serum concentrations (Fig. 2A). There was also a concentration ratio of about 4.5:1 between follicular fluid and serum, indicating active uptake by the follicle against a concentration gradient [42, 69]. This would be consistent with the sequestration of ascorbate to facilitate rapid follicular expansion during the approach to ovulation and/or post-ovulatory steroidogenesis. A similar but lower gradient evidently exists across the bovine follicle [70]. Our data also reveal a strong correlation between follicle volume and the serum ascorbate concentration (Fig. 2B), suggesting that follicle growth, particularly during the late stages of exogenous stimulation, may be limited by the availability of ascorbic acid in the circulation. The mechanism of uptake of ascorbate by the ovary has yet to be determined, but leukocytes are closely associated with the ovarian tissue cycle [71] and may provide a locally concentrated source.

As with males, vitamin C has been suggested as a regulator of female fertility. Ascorbic acid supplementation enhanced the ovulation-inducing effects of clomiphene by an apparently local ovarian effect [72], and an early study reported conception in otherwise infertile cows after ascorbic acid administration at estrus [47]. In contrast, a contraceptive action of high doses of ascorbate has been proposed, based on alterations to the structure of cervical mucus [50]. Large quantities of ascorbic acid are utilized during human conception [73] and are necessary to maintain the integrity of the fetal membranes [74]. Dietary supplementation during pregnancy may reduce the frequency of birth defects [75], and a daily supplement of at least 500 mg of vitamin C, starting as early in pregnancy as possible, has been suggested for pregnant mothers [73]. As yet, none of these proposals has been rigorously examined or translated into clinical practice.

CONCLUSION

The three primary biological functions of ascorbic acid (collagen synthesis, hormone secretion, and anti-oxidation)

may explain many of the known effects of the vitamin on reproduction. 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 at several levels in the reproductive process. In domestic species and other mammals capable of synthesizing ascorbate, 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 life style) become variables in the multifactorial equations of fertility. The available human data suggest that a reexamination of the clinical potential of vitamin C would be timely. A complete understanding of the ascorbate dependence of reproduction is of immediate practical interest to us as regulators of our own fertility.

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