

Full Length Research Paper

The effects of high level ascorbic acid on pregnancy rate and litter size in rats

Muhterem Aydin* and Murat Yuksel

Department of Obstetric and Gynecology, Faculty of Veterinary, University of Firat, 23119 Elazig, Turkey.

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The effects of high dose ascorbic acid on pregnancy rate and litter size in rats were investigated in this study. For this purpose, forty rats were used. The animals were randomly divided into four groups as control (n = 10) and treatment (n = 30). The rats were randomly assigned to three groups in treatment group. Within this group, 15 females and 15 males were used. Females in Groups 1 and 3, and males in Groups 1 and 2 were administered 1.5 g/kg body weight vitamin C high dose ascorbic acid orally, in drinking water, freshly prepared daily for 15 days. The results showed that the use of high dose ascorbic acid in male Groups (1 and 2) caused an insignificant increment on pregnancy rate and litter size when compared to the control groups and Group 3. Thus, the use of high dose ascorbic acid may reduce fertilizing capability of sperm. On account of this, pregnancy rate may be falling away.

Key words: Rat, ascorbic acid, pregnancy rate, litter size.

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 (Adul-Aziz et al., 1995) but its precise physiological role in reproduction has been uncertain. Recent data suggests that ascorbate has defined functions in hormone secretion, gamete protection, and gonadal tissue remodeling. Its effects can therefore be explained by cellular and biochemical mechanisms similar to those applicable in other tissues. L-isomer of ascorbic acid, known as vitamin C, which is a water-soluble vitamin required for normal function of the body. 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 (Zreik et al., 1999; Luck et al., 1995).

It is widely used for the scavenging of free radicals, strengthening the immune system and prevention of chemical carcinogenesis induced by a number of xenobiotics (Head et al., 1998; Turk et al., 2008). It has also been reported that ascorbic acid has direct antiviral properties and stimulates interferon production by virus-infected cells in culture (Fettman, 2001).

Early studies reported direct effects of ascorbate deficiency on male fertility in laboratory and farm species. Low or deficient ascorbic acid levels have been associated with low sperm count, increased numbers of abnormal sperm, reduced motility, and agglutination (Luck et al., 1995). Whereas high ascorbic acid levels have been inhibition of hyaluronidase. Hyaluronidase is one of the acrosomal enzymes and it is required for the penetration of sperm through the cumulus oophorus matrix during the fertilization. This enzyme is released from the head of sperm during acrosomal reaction and degrades hyaluronic acid, a glycosaminoglycan present in the extracellular matrix of ovum (Lin et al., 1994; Tanyildizi and Bozkurt, 2004; Millar, 1992).

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

*Corresponding author. E-mail: muhteremac@gmail.com Tel: +904242370000/3858. Fax: +904242388173.

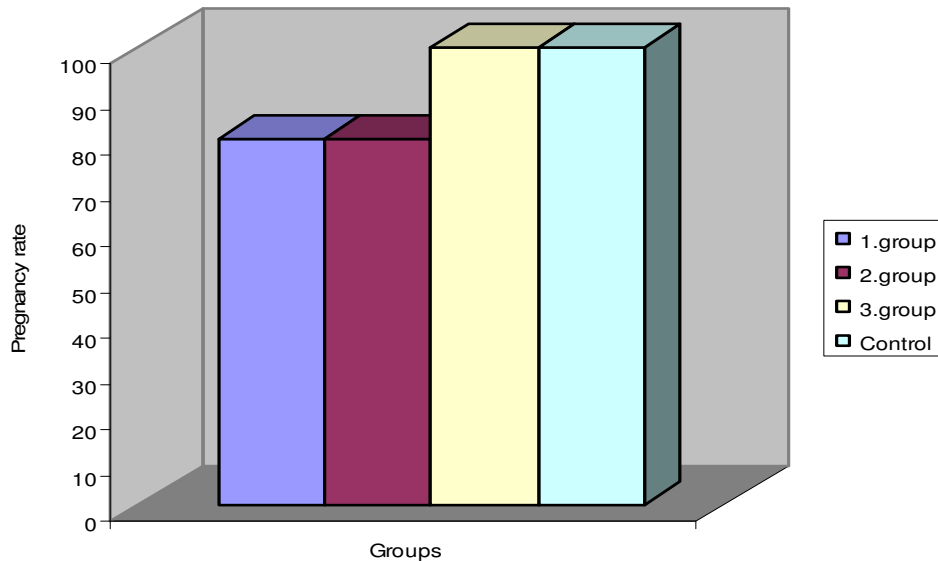


Figure 1. The effects of high dose ascorbic acid on pregnancy rate.

luteal compartments. More recent studies with luteinizing granulosa cells show that ascorbate is stimulatory to progesterone and oxytocin secretion (Luck et al., 1988), consistent with its known roles in hormone biosynthesis, and synergizes with neurotransmitters in stimulating hormone secretion. 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. 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. Collagen synthesis is required for follicle growth, for repair of the ovulated follicle, and for corpus luteum development. Ascorbate will also be needed for secretion of collagen and proteoglycans into follicular fluid (Luck et al., 1995, 1988; Zreik, et al., 1999). The goal of the study is to investigate the possible adverse effects of high dose ascorbic acid on pregnancy rate and litter size in Wistar rats.

MATERIALS AND METHODS

In the present study, a total of 40 Wistar rats, with 20 females aged 4 to 6 months, weighing 200 to 250 g and 20 males weighing 250 to 300 g were used. Animals were maintained under standard laboratory conditions on a 12 h light to 12 h dark cycle in a temperature-controlled room at 21 to 22°C. The rats were housed in cages specially designed to minimize field perturbation. The size of the cages was similar to that of commercially available rat breeding cages, (33 x 13 x 15 cm). The walls of the cages consisted of perspex, and feeding pens and water bottles were mounted outside the cages. The rat had free access to maintenance food and water. Commercially obtained cork flakes were used as bedding material. The cages were washed once a week. The investigation was made with the permission of the Ethical Committee on Animal Experiments of the Fırat University.

The rats were assigned to experimental and control groups. The rats were randomly assigned to three groups in experimental group. Within this group, 15 females and 15 males were used. Females in Groups 1 and 3, and males in Groups 1 and 2 were administered 1.5 g/kg vitamin C (L-ascorbic acid (Redoxon®) was obtained from Roche (Roche Inc., Istanbul, Turkey) orally, in drinking water, prepared fresh daily for 15 days. Group 2 females and Group 3 males were administered only water. The end of administered ascorbic acid animals in all groups were mated. The day sperms were detected in the vaginal smear was determined as the 0th day of pregnancy. On day 18, female rats were sacrificed and assessed for pregnancy rate and litter size.

RESULTS

The effects of high dose ascorbic acid on pregnancy rate and litter size were given Figures 1 and 2. The pregnancy rate in Groups 1, 2 and 3 and control were determined as 80, 80, 100 and 100%, respectively. It was found that pregnancy rate was insignificant decreased in Groups 1 and 2 compared with the control group and Group 3. The litter size was not effected in all groups. It was concluded that the pregnancy rate insignificant increment in the experiment Groups 1 and 2 compared to the control group and Group 3.

DISCUSSION

Ascorbic acid is required for many biochemical reactions in the body. This vitamin plays an important role in male and female fertility. The concentration of ascorbic acid in semen and ovary are 8 to 10 folds higher than that in blood (Jacob et al., 1992) and it is extremely sensitive to a decrease in blood levels of ascorbic acid (Chinoy et al.,

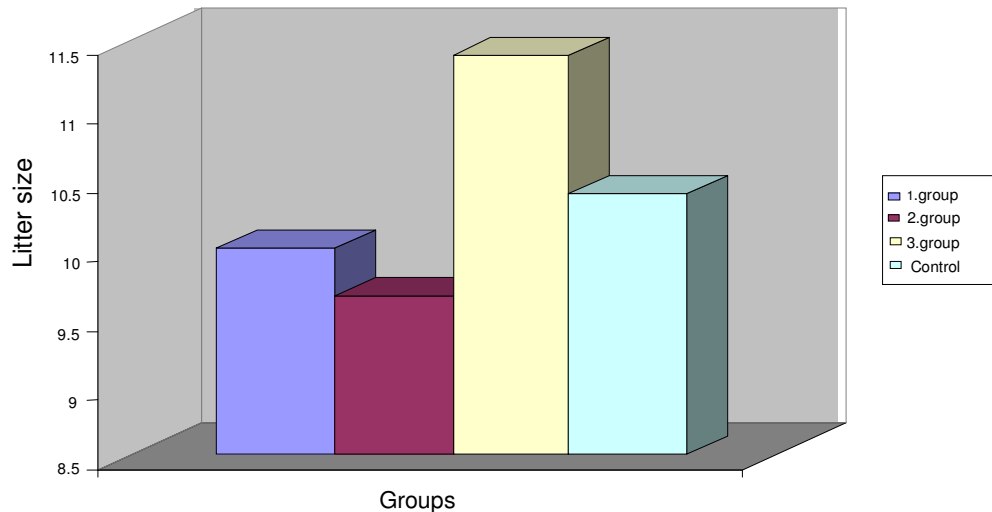


Figure 2. The effects of high dose ascorbic acid on litter size.

1986). Ebesunun et al. (2004) indicated that semen ascorbate level may play a significant role in male fertility, and the decrease in concentration of ascorbic acid of semen caused to reduce sperm characteristics. Similarly, some early studies demonstrated that the deficiency of ascorbic acid caused degeneration of the testicular germinal epithelium (Gomes et al., 1977) reduced sperm concentration and motility (Cieresko et al., 1995) and, associated with poor breeding performance (Phillips et al., 1940). Furthermore, male fertility would be improved by an increased dietary ascorbic acid intake (Dawson et al., 1987). Sonmez et al. (2003) indicated that although short-term administration of the ascorbic acid increased ascorbic acid level of semen within 24 h after treatment, it did not affect the sperm characteristics. However, the administration of ascorbic acid along 15 days caused an improvement in semen quality of rams. The results of present study found that administration of ascorbic acid for 15 days insignificantly decreased pregnancy rate. The results of this result can be explained by the along-term administration of ascorbic acid in this study.

Hyaluronidase is one of the acrosomal enzymes. This enzyme is released from the head of sperm during acrosomal reaction. The hyaluronidase is required for penetration of sperm through cumulus oophorus matrix during fertilization (Lin et al., 1994; Tanyildizi and Bozkurt, 2004). Abdul-Aziz et al. (1995) suggested that the inhibition of hyaluronidase activity could be a simple mechanical block to sperm penetration and might reduce fertilization rate. Li et al. (2001) suggested that some flavonoids such as kaempferol, quercetin and apigenin inhibited the sperm hyaluronidase activity and sperm penetration. Similarly, it was reported that the antifertility effects of some drugs such as sodium aurothiomalate (Perreault et al., 1980) and gossypol (Yuan et al., 2000) might be due to the inhibition of hyaluronidase.

Kramer et al. (1933) suggested that ascorbic acid plays a vital role in follicular development as scorbutic guinea pigs were shown to be an ovulatory with marked follicular degeneration and these animals also had increased rates of implantation failure, and increased spontaneous abortions. More recent studies have shown that ascorbic acid blocks apoptosis in cultured follicles, which led Tilly and Tilly (1995) to the conclusion that ascorbic acid and other antioxidants serve a role to prevent atresia. Behrman et al. (1996), showed that ascorbic acid transport is up-regulated by follicle stimulating hormone (FSH) and IGF-I and that antioxidants block the spontaneous resumption of meiosis in rat oocytes removed from the follicular environment.

The results of the present study indicated that the administration of high dose ascorbic acid for 15 days insignificantly decreased pregnancy rate. The findings of this study indicated that high dose L-ascorbic acid may have caused a significant decrease in semen hyaluronidase activity.

Hyaluronidase enzyme is considered to be involved in many physiological and pathological processes like fertilization, tumor growth and metastasis. Bacterial hyaluronidases contribute to the spreading of microorganism in tissue. In this regard, ascorbic acid could be useful as pharmacological drug to prevent certain functions of hyaluronidase. However, hyaluronidase which localizes in the acrosomal region of sperm plays an important role at fertilization and the reduction of its activity in the acrosome may cause a decrease in fertilizing capability of sperm (Hirayama et al., 1989). Therefore, administration of ascorbic acid may cause to inhibition or reduction of hyaluronidase activity and, prevent sperm penetration into cumulus complex.

Hyaluronidase is required for the penetration of sperm through the cumulus oophorus matrix during the

fertilization. Administration of ascorbic acid may prevent sperm penetration into cumulus complex due to reduction or inhibition of hyaluronidase activity in the long term, and it can cause a decrease in fertilization rate. However, further studies are required to examine whether there is relationship between high dose ascorbic acid and long term treatment on fertilization.

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