INTRODUCTION

The purpose of teratogenic screening methods is to establish experimental criteria by which one could predict that a drug given during pregnancy under various environmental conditions is likely to exert, or not, adverse effects in the embryo. The action of a drug on the embryo is often completely different from that produced by the same compound in the adult. Embryos usually have a higher susceptibility to drugs than adults. This susceptibility varies not only between different animal species, but also within a given species between different strains and even between individuals of the same strain. The teratogenic action depends much more upon the specific susceptibility of the embryo than upon the toxicity and the particular pharmacological activities of the drug. Even non-toxic compounds, perfectly well tolerated by the mothers, can be harmful to the developing embryo and produce malformations. The type of malformation induced by the teratogen is dependent not only on the nature and dosage of the agent but also to a large extent on the precise developmental stage of the embryo and the physiological or pathological state of the mother **(Frohberg, 1977)**.

Experiments performed in the field of teratology led to the discovery of a great number of teratogenic agents that produce congenital malformation. Several types of drugs, which may be used during pregnancy, have been found to induce teratogenicity in human and laboratory experimental animals **(Schardein, 1977)**.

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting about 1% of the population world wide with preference to women between the ages of 20-50. RA is heterogeneous disease characterized by a wide spectrum of severity ranging from mild to a

severe form such as synovitis which causes cartilage destruction, bone erosions and joint deformities leading to physical disability, pain, fatigue, and increased mortality. The cells involved in the synovial inflammation are T cells, macrophages, synoviocytes, dendritic cells and plasma cells **(Goldenberg, 1999; Herrmann *et al.*, 2000; Pap *et al.*, 2003; Bovin *et al.*, 2004; Simon, 2004; Vergne-Salle *et al.*, 2005; Aguillón *et al.*, 2006 and Litinsky *et al.*, 2006).**

Antirheumatic drugs may have a negative effect on reproduction in both men and women. Possible negative effects are impairment of fertility and harmful effects on the foetus **(Østensen, 2006).** Issues regarding drugs and reproduction are not always sufficiently discussed with female and male patients **(Østensen *et al.*, 2007).** Leflunomide **(LEF)** is a disease modifying antirheumatic drug (DMARD). The actual wording in the label of **LEF** with regard to the reproductive warnings is as follow: "There are no adequate and well controlled studies evaluating **LEF** in pregnant women. Upon discontinuing **LEF**, it is recommended that all women of childbearing potential undergo the drug elimination procedure. Women receiving **LEF** and who wish to become pregnant must discontinue **LEF** and undergo the drug elimination procedure". In spite of these warnings approximately 30 women have become pregnant while taking **LEF** as of December 1999 **(Brent, 2001).** In addition, 43 pregnancy outcomes with first-trimester exposure to **LEF** have been published in abstract **(Chambers *et al.*, 2004)** and 13 case reports of **LEF** exposure during pregnancy were reported by **De Santis *et al.* (2005).** The main reason that directs women to take **LEF** during pregnancy is the chronic nature of RA which means that patients require drug therapy for many years **(Van der Heijden *et al.*, 2007).**

The current study is aiming to investigate the effect of **LEF**, an antirheumatic drug, on mice foetuses; 20th day of gestation. This study is comprised of:

- Morphological examination of the foetuses obtained.
- Histological and histochemical studies to elucidate the normal structure, histopathological changes and the variations in DNA and protein contents of foetal liver.
- Extracting the total DNA genome of the foetuses of the normal and drug treated groups, amplification of certain nuclear gene (cytochrome oxidase subunit VIII; **COX8**) by using the polymerase chain reaction, digesting the chosen gene by using several restriction endonuclease enzymes and making molecular foetuses toxicity genotypes according to their total DNA and chosen restriction fragment patterns.
- Bioinformatics is used to detect some mutations that occurred in the **COX8** genes of the foetuses of the drug exposed groups with low effort and cost. Also, the use of the bioinformatics has been extended to predict the RNA secondary structures and draw the phylogenetic trees that easily show the extent of convergence between foetuses of the different studied groups. In addition other parameters were also studied.