Tamoxifen, a nonsteroidal antiestrogen, is the endocrine treatment of choice for all stages of breast cancer in pre- and postmenopausal women. Estrogens stimulate the proliferation of target cells by transcriptional mechanisms after binding to the estrogen receptor. The drug tamoxifen competes with estrogen for binding to the receptor. In this thesis the effects of tamoxifen on the female genital tract and psychosexual functioning are studied.

After a general introduction, in chapter 1 a review is given of the effects of tamoxifen on the female genital tract including psychosexual effects. The English-language literature in MEDLINE and reference lists from selected articles were used, with special interest in differentiation of side effects in premenopausal and postmenopausal women. The most frequently reported side effect was hot flashes, and the most discussed side effect was the increased endometrial cancer risk. Weighted estimates of severeness and extent of side effects were mostly not possible because of lack of randomized trials. Only the risk of endometrial cancer in relation to tamoxifen treatment could be estimated, being two- to three-fold. The gynecologic side effects of tamoxifen are diverse and reflect the complexity of its mechanism of action, with agonistic and antagonistic effects on various tissues, depending on the ambient serum estradiol concentration and hence menopausal status of the patient. The issue of endometrial surveillance and possible options for screening and intervention to abate side effects are discussed.

In chapter 2 the clinical implications of the effects of tamoxifen on the postmenopausal endometrium are discussed. In chapter 2.1 we describe the tamoxifen effects on the endometrium in postmenopausal breast cancer patients by transvaginal ultrasonography (TVU), hysteroscopy and histology of endometrial samples. Out of 53 asymptomatic postmenopausal breast cancer patients 58% had an increased endometrial thickness and abnormal appearance of the endometrium on TVU, suggesting endometrial pathology. Hysteroscopy revealed endometrial polyps in 22% of them, histologically characterized as glandulocystic atrophy, with dilated glands and dense stroma. However, in the majority of the patients no hysteroscopic or histologic explanation for the thickened endometrium on TVU could be found. In three hysterectomy specimens, histology of the total endometrial layer showed a similar glandulocystic atrophy as was found in the polyps and these findings explain the discrepancy between TVU and hysteroscopy/histology. Due to the high number of false-positive findings, we conclude that TVU is not an effective screening instrument for the endometrium in postmenopausal tamoxifen users.

Chapter 2.2 describes a case report of a patient who developed adenosarcoma of the uterus after two years of adjuvant tamoxifen treatment for breast cancer. The possible association between tamoxifen treatment and uterine sarcoma is discussed and the literature is reviewed.

In chapter 2.3 a contribution to the discussion concerning the usefulness of endometrial surveillance in tamoxifen users, in response to the studies of Barakat et al. and Love et al. is given. We discuss the explanation for the discrepancy between TVU versus hysteroscopy and histology, and the high false-positive rate.
Summary

of ultrasonographic findings i.e. the characteristic endometrial stromal and epithelial reactions to tamoxifen as described in chapter 2.1. We recommend that all tamoxifen treated patients should be instructed and encouraged to report to their doctor abnormal vaginal bleeding or discharge. Only those patients who present with symptoms should have a hysteroscopy with endometrial sampling, to exclude or diagnose endometrial cancer.

In chapter 3 tamoxifen effects on epithelial proliferation and estrogen receptor and progesterone receptor expression were analyzed in 125 samples of postmenopausal endometrium. In benign endometrial epithelium, the proliferation index by Ki-67 expression was higher in tamoxifen users (13%) than in non-users (2%). In endometrial cancer the proliferation index was higher than in benign endometrium, but similar in tamoxifen users (32%) and non-users (35%). Estrogen receptor expression was comparably high in benign epithelium from tamoxifen users (97%) and non-users (92%), but lower in endometrial cancer occurring in tamoxifen users (60% vs. 88%). In stromal cells the progesterone receptor expression was higher in tamoxifen users, both in benign (84% vs. 54%) and malignant endometrium (33% vs. 10%). The observed higher proliferation rate in endometrial epithelium from tamoxifen users might play a role in the observed higher incidence of endometrial cancer in tamoxifen users. The higher progesterone receptor expression in tamoxifen exposed stromal cells suggests an estrogenic effect on the endometrial stroma, independent of the histologic diagnosis.

In chapter 4 apoptosis and apoptosis-associated parameters in the endometrium of tamoxifen-exposed and non-exposed women are studied. Endometrial tissue samples from 125 postmenopausal women with benign or malignant endometrium with and without tamoxifen-exposure were examined. Apoptosis was evaluated morphologically by light microscopy and the percentage of apoptotic cells was expressed as the apoptotic index (AI). In addition, in benign endometrium from tamoxifen-users and non-users, epithelial cell apoptosis was evaluated by the expression of caspase-3 and by a neo-epitope on cytokeratin 18, unmasked by an early caspase-cleavage event and recognized by the monoclonal antibody M30. Expression of apoptosis-associated parameters such as Fas, FasL and Bcl-2 were evaluated in all samples. The AI in benign endometrium was 0.17% in tamoxifen-users and 0.08% in controls, and in endometrial cancer 2.49% in exposed and 2.26% in non-exposed endometrium. In benign endometrium, apoptosis by the expression of caspase-3 and M30 reactivity was absent in tamoxifen-users and low or absent in controls and did not correlate with the AI, Fas, FasL or Bcl-2 expression. Correlation of the AI with the previously reported Ki-67 index in benign endometrium showed that the AI/Ki-67 index was lower in tamoxifen-users (0.02) than in non-users (0.05) (p<0.005). FasL expression was more frequently expressed in benign epithelium from tamoxifen-users than in controls (40% vs. 9%, p<0.05), while no changes in the expression of Fas and Bcl-2 in benign or malignant endometrium were observed by tamoxifen-exposure. In
conclusion, in postmenopausal, benign endometrium, the AI/Ki-67 index in tamoxifen-users is lower than in controls, indicating an imbalance between cell death and cell proliferation, in favor of proliferation, together with an increased FasL expression. These findings imply an imbalance in the apoptosis/proliferation equilibrium and possibly immunology, in tamoxifen-exposed endometrium, that might contribute to the increased endometrial cancer risk in postmenopausal tamoxifen-users.

In *chapter 5* patient-related parameters determining ovarian cyst formation in women using tamoxifen for breast cancer were evaluated. We performed a cross-sectional study in 142 pre- and postmenopausal breast cancer patients using tamoxifen. Gynecological assessment, transvaginal ultrasonography (TVU) and serum estradiol and follicle stimulating hormone analysis were performed. After the initial examination, follow-up assessments were performed twice a year. Unilateral or bilateral ovarian cysts were detected by TVU in 24 tamoxifen-using patients. Multiple regression analysis showed that cyst development is related to high serum levels of estradiol, younger age and absence of high-dose chemotherapy, indicating a premenopausal status of the patient. No patients older than 50 years of age, after high-dose chemotherapy or with amenorrhea over one year and low levels of estradiol, developed ovarian cysts. Patients still having a menstrual cycle during tamoxifen had a high chance (over 80%) of developing ovarian cysts. Thus, breast cancer patients receiving tamoxifen only develop cysts if their ovaries are able to respond to ovarian stimulation by a high estradiol production.

In *chapter 6* the impact of tamoxifen on subjective and psychosocial well-being in relation to type of prior chemotherapy and menopausal status was studied. Patients participated in a randomized study comparing adjuvant standard-dose and high-dose chemotherapy in breast cancer patients under 56 years of age, followed by two years of daily 40 mg tamoxifen. Every six months during tamoxifen treatment and once after cessation, a structured interview was performed, containing items of physical, mood-related and psychological symptoms associated with tamoxifen and menopause. During tamoxifen the most frequent complaints were hot flashes, disturbed sleep, vaginal dryness, dyspareunia, decreased sexual desire and musculo-skeletal symptoms. Disturbed sleep correlated with hot flashes and concentration problems while decreased sexual desire correlated with vaginal dryness and dyspareunia. In the high-dose arm all except one patient became postmenopausal and more patients reported symptoms than in the standard-dose arm. After discontinuation of tamoxifen, symptoms decreased. However, hot flashes, disturbed sleep and vaginal dryness persisted more often in patients who had become postmenopausal due to (high-dose) chemotherapy.