

ENDOMETRIAL CARCINOMA IN THE RABBIT AS AN ANIMAL MODEL FOR HUMAN ENDOMETRIAL
CARCINOMA WITH SPECIAL REFERENCE TO SOME ENDOCRINE FEATURES

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Introduction

Compared to man endometrial carcinomas in animals are uncommon, except in cows (6), in a recently described colony of virgin Han:Wistar rats, and in rabbits. In the mentioned colony of virgin Han:Wistar rats 119 (39%) out of 305 females developed endometrial carcinomas (8). According to the literature, mainly based on observations on large colonies of laboratory rabbits during many years, the incidence is varying from 15% to 50% of all spontaneous died animals (2,5,13,18). The endometrial carcinoma in rabbit has been recommended as an animal model for human disease (4). This paper presents the main characteristics of endometrial carcinomas in rabbits, especially with regard to the suitability of this tumour as an animal model for human disease.

Material and Methods

The investigation concerned two groups of rabbits (group A and group B). Group A consisted of 150 female laboratory White Viennese x Alaska rabbits which were allocated to 5 groups of 30 animals each for a life-span feeding experiment. The rabbits were housed individually. There were no male rabbits in their neighbourhood. The rabbits were never mated. They were given a semi-synthetic diet supplying 25% of the energy as fat. The dietary fats had divergent fatty acid compositions (27). Almost all rabbits died spontaneously at an age varying from 18 months to 10 years. All animals were necropsied.

The macroscopically discovered tumours, the metastases and the non-tumorous parts of the uteri with tumours were fixed in 4% formalin. After embedding in paraffin, sections of 6 micrometres were stained with haematoxylin and eosin, van Gieson, periodic acid Schiff and Sudan black.

Group B consisted of 50 breeding rabbits. Thirty animals of this group were Alaska and 20 were White Viennese rabbits. From an age varying from 2 to 3 years these rabbits were housed individually during 2 to 5 years. During this period the rabbits were never mated and there were no male rabbits in their neighbourhood. In 5 of the developed endometrial carcinomas estrogen receptor (ER) and progesterone receptor (PR) were estimated using a dextran-coated charcoal technique. For determination of the ER the cytosols were incubated at 4°C for 16 hours with ³H-17β-oestradiol. Unlabelled diethylstilboestrol (DES) was used to correct for non-specific binding sites. For determination of the PR the cytosols were incubated with ³H-Org-2058, a synthetic progesterone-derivate. Unlabelled Org-2058 was used to correct for non-specific binding sites. The ER and PR concentrations and the dissociation constants were determined from a plot of the data according to Scatchard(23). In 16 cases of endometrial carcinomas occurring in group B subcutaneous transplantation experiments were performed using fragments of tumour tissue measuring less than 0.5 mm in diameter. Five endometrial carcinomas were used for tissue culture experiments.

Results

Group A

The total number of endometrial carcinomas found on post mortem examination was 80 (54%) out of 150 female rabbits. There were no significant differences in incidence between the 5 dietary groups. The incidence of endometrial carcinomas increased with age, reaching 67% in animals over 4 years of age. Thirty-three (44%) of the carcinomas had metastasized, mostly to the lungs. Fig. 1 shows the incidence of endometrial carcinomas and the number of metastases at different ages. In most cases the endometrial carcinomas were primary multiple and present in both uterine horns. The histological structure of the tumours varied greatly in differentiation grade. In the stroma of some of the carcinomas groups of cells were found resembling the foam cells as described in endometrial carcinomas and in glandular hyperplasia in women. These mesenchymal cells are characterized by foamy Sudan black positive cytoplasm. The non-tumorous parts of the uteri with carcinomas often showed cystic endometrial hyperplasia or atrophy of the endometrium. Sometimes both changes were found in several parts of the same uterus.

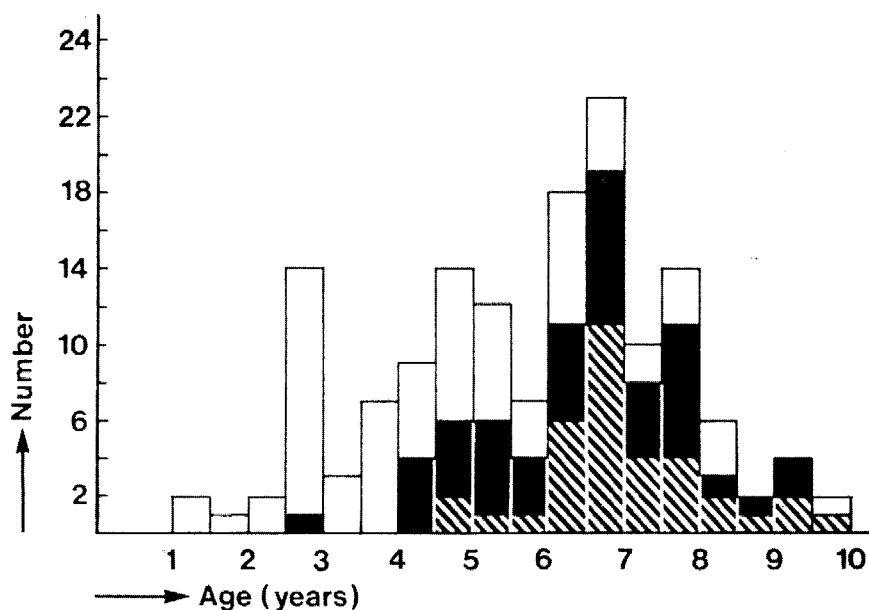


Fig. 1. Incidence of endometrial carcinomas and number of metastases at different ages.

White columns (down to the x-axis): examined rabbits.

Black columns (down to the x-axis): rabbits with carcinomas.

Shaded columns: rabbits with metastasized carcinomas.

Group B

Within a period of 5 years of individually housing of the rabbits, 25 out of the total group of 50 rabbits developed endometrial carcinomas. There was no significant difference in incidence between the Alaska and the White Viennese rabbits. The macroscopical as well as the microscopical findings in these carcinomas corresponded with those of group A.

The 5 examined endometrial carcinomas were ER as well as PR positive. Table I shows the ER and PR values and the ER and PR dissociation constants.

ER (fmol/mg protein)	ER-Kd ¹ (10 ⁻¹⁰ M)	PR (fmol/mg protein)	PR-Kd ² (10 ⁻¹⁰ M)
125	10	60	6.2
150	2.5	310	3.8
165	7.3	195	7.6
340	2.7	290	3.3
375	3.9	315	5.8

Table I. Estrogen and progesterone receptors and their dissociation constants in 5 endometrial carcinomas in rabbits.

¹) ER-Kd = the dissociation constant of the estrogen receptor.

²) PR-Kd = the dissociation constant of the progesterone receptor.

Successful autologous subcutaneous transplantation was effected in 11 of 16 rabbits with endometrial carcinomas (fig. 2).

Five endometrial carcinomas were used for tissue culture experiments. These 5 tumours could be cultured (cell and tissue culture) using as tissue culture medium Ham's F-12 and Leibovitz L-15 (fig. 3).



Fig.2. Growth of endometrial carcinoma (at arrows) after autologous subcutaneous transplantation of fragments of tumour tissue measuring less than 0.5 mm. in diameter. Rabbit.

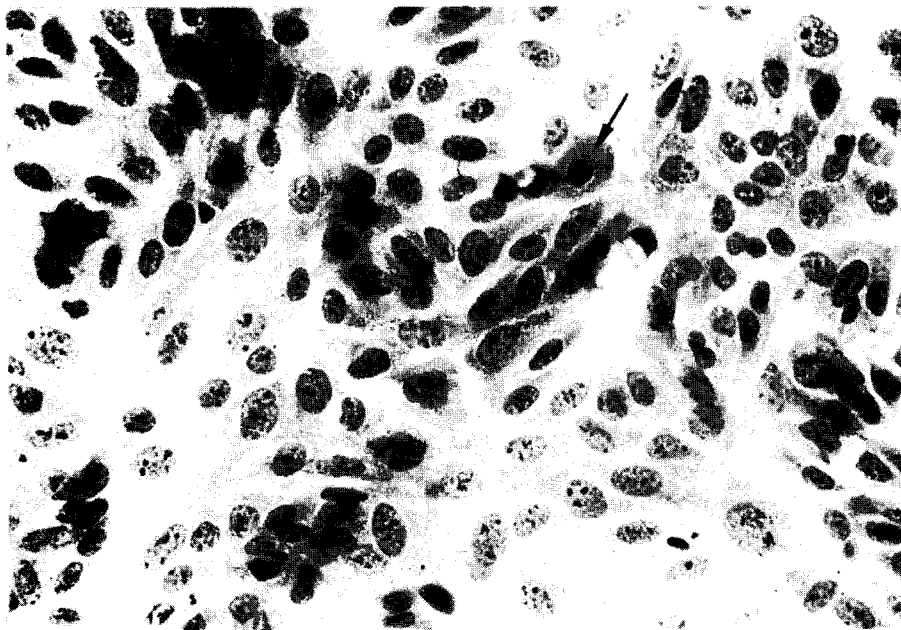


Fig.3. Tissue culture of rabbit endometrial carcinoma cells. Mitotic figure at arrow. Haematoxylin. Obj. 25

Discussion

The very high incidence of endometrial carcinomas in our colony of laboratory rabbits (group A), especially in rabbits over 4 years of age, is in agreement with previous studies (2,5,15,18). Most of the larger series of rabbits with endometrial carcinomas are laboratory animals. Possibly this is caused by the fact that many laboratory rabbits are almost continuously in oestrus, while non-laboratory rabbits are often pregnant, lactating or in anoestrus (1). In the latter conditions follicular activity (oestrogen) is lower than in rabbits which are in oestrus. The rabbits of our colony with their high incidence of endometrial carcinomas were also housed individually and they were never mated. As the rabbit is an induced ovulator, they were never in the luteal phase, but almost continuously in oestrus. The literature with regard to the association between endometrial carcinomas and hyperoestrogenism in rabbits is controversial, as are the data referring to women. There are several arguments in favour of such an association.

Signs of excessive oestrogen action in laboratory rabbits with endometrial carcinomas are noted (15).

Also in agreement with the hypothesis that hyperoestrogenism is a factor in the development of endometrial carcinomas in rabbits is the high incidence of endometrial carcinomas in old breeding families of some colonies of laboratory rabbits after toxæmia of pregnancy (13). This might be explained by hyperoestrogenism as a result of impaired liver function (13).

Moreover the induction of endometrial carcinomas in rabbits by application of oestrogens is reported (3,20).

The frequent finding of cystic endometrial hyperplasia, an abnormality which is in rabbits related with hyperoestrogenism (5,12,20), in the non-tumorous parts of the uteri with endometrial carcinomas is another argument in favour of an association between endometrial carcinomas and hyperoestrogenism in rabbits. The frequent finding of cystic endometrial hyperplasia in uteri with endometrial carcinomas is in agreement with the literature (5,13). There are signs that there is an evolutionary scale from benign cystic endometrial hyperplasia to malignant anaplastic carcinoma (5,21). Also in woman endometrial hyperplasia is presumed to be a precursor of endometrial carcinoma (17). Compared to woman our finding of groups of "foam cells" in the stroma of some of the endometrial carcinomas also suggests a relationship between endometrial carcinomas and hyperoestrogenism in rabbits. In woman the presence of "foam cells" in the stroma of endometrial carcinomas is reported to be associated with hyperoestrogenism (7).

There are also arguments which seem to be in contradiction with an association

between endometrial carcinomas and hyperoestrogenism in rabbits.

Some of the endometrial carcinomas in our colony of rabbits as well as some of those reported in literature (4) were found in uteri with atrophy of the endometrium instead of the above mentioned cystic endometrial hyperplasia. However in some of our cases endometrial atrophy and cystic endometrial hyperplasia were found in several parts of the same uterus.

A second argument in contradiction with an association between endometrial carcinomas and hyperoestrogenism in rabbits seems to be the finding that administration of oestrogen to aged Dutch rabbits reduced the incidence of endometrial carcinomas from 17% to 3% (4).

In our opinion the arguments in favour seem to be more valid than those against an association between endometrial carcinomas and hyperoestrogenism in rabbits.

The successful autologous subcutaneous transplantation is in agreement with previous findings (15).

Based on the following characteristics the endometrial carcinoma in the rabbit appears to be a suitable animal model for human endometrial carcinoma. The incidence is very high. The endometrial carcinomas in rabbits can be studied by autologous subcutaneous transplantation, for instance with regards to the effects of hormonal and chemotherapeutical agents on the growth of the tumours. Some characteristics of the rabbit endometrial carcinomas can be studied in vitro. These tumours grow very well in tissue culture. In rabbits (1,3,5,12,13,14,15,20) as well as in women (7,9,11,19,22,24,25) the incidence of endometrial carcinomas have been associated with hormonal dysfunction. Based on the presence of ER and/or PR in the tumour tissue (a number of) the endometrial carcinomas in rabbits are to be called hormone dependent. Also in women endometrial carcinomas often are ER and/or PR positive (22,26). Further studies on the significance of the presence of ER and PR in the tumours and on the role of hormonal factors in the pathogenesis of endometrial carcinomas in rabbits, worthwhile from the comparative (model) point of view, are in progress (10).

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Summary

A description is given of the carcinomas occurring in a group of 150 White Viennese x Alaska rabbits. The rabbits were observed from half a year till death. Almost all rabbits died spontaneously. The incidence of the endometrial carcinomas was 54% (80 cases). Thirty-five (44%) of the carcinomas had metastasized, mostly to the lungs. The tumours varied greatly as regards the differentiation grade. A possible association between endometrial carcinomas and hyperoestrogenism in rabbits is discussed.

A second group of rabbits with endometrial carcinomas was used for estimation of estrogen and progesterone receptors in the tumour tissue and for transplantation and tissue culture experiments. The 5 examined endometrial carcinomas were estrogen as well as progesterone receptor positive. Successful autologous subcutaneous transplantation was effected in 11 of 16 rabbits with endometrial carcinomas. The endometrial carcinomas could easily be cultured. The endometrial carcinoma in the rabbit appears to be a suitable model for human endometrial carcinoma.

