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### Possibility of Freemartinism in Roe Deer <sup>1</sup>

MOŻLIWOŚĆ WYSTĘPOWANIA FRYMARTYNIZMU U SARNY

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Sysa P. S. & Kałuziński J., 1984: Possibility of freemartinism in roe deer. Acta theriol., 29, 10: 133—137 [With Plate I]

Cytogenetic analysis was made in female roe deer, *Capreolus capreolus* (Linnaeus, 1758), belonging to members of heterosexual triplets. Lymphocytic chimerism was not discovered, the karyotype being defined as 70,XX. The result obtained justifies the assumption that in cases of twin or multiple pregnancy of unlike sexes in the roe deer there is no development of infertility in the females etiologically similar to the freemartinism syndrome in domestic cattle.

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## I. INTRODUCTION

The freemartinism syndrome observed in domestic ruminants (particularly in cattle, and sporadically in goats and sheep) develops in females born from twin pregnancies when their co-twin during the embryonic period is a male (Lillie, 1916; Balbierz, 1971; Jost *et al.*, 1973a; Bielańska-Osuchowska, 1977). This phenomenon consists in the placentas of the embryos connecting by means of vascular anastomoses. In consequences of this connection motile embryonic tissues, particularly haemopoietic tissues, migrate between the embryos. It has been assumed that gonocytes also migrate in this way. At the same time reciprocal reaction on each other of hormonal and immunological systems takes place in the twins of the embryonic period, causing disturbance of the formation of gonads and elements of the reproductive system originating from Müllerian ducts in the female embryo (Jost *et al.*, 1973; Sysa *et al.*, 1980). The effect of these processes consists in various kinds of defects in development and structure of the genital system in females, usually leading to their infertility. In domestic cattle the basic cause of the phenomenon described above is the failure to form of the inter-placental barrier in such cases.

This phenomenon has not been diagnosed in wild ruminants, this including the roe deer, which in this respect is a peculiar species, as it has been found that twin and multiple pregnancies occur in as many as 94% of the females (Strandgaard, 1972a). In the experimental hunting area at Czempin, about 82% of the young originate from bigeminal and multiple pregnancies (Kałuziński, 1982). Similar results to the above have been obtained by other researchers. A considerable percentage of such pregnancies (about 50%) consist of embryos of unlike sex (Borg, 1970; Strandgaard, 1972b). It is therefore necessary to ascertain whether a developmental syndrome similar to freemartinism in domestic ruminants forms in the roe deer.

The purpose of the present study was to determine whether the phenomenon of lymphocytic chimerism, which is an indicator of the formation of vascular placental fusions, takes place in females of the roe deer from multiple pregnancies in which a male was a partner of the embryonic period.

## 2. MATERIAL AND METHODS

The study material consisted of blood samples collected from two female roe deer *Capreolus capreolus* (Linnaeus), 1758) born from multiple pregnancies. Both the females originated from litters consisting of three fawns, as follows: 2♀,♂;♀ 2♂. The fawns were born in the experimental enclosure of the Polish Hunting Association Research Station at Czempin (Poznań voivodship).

Blood was sampled in vivo from the jugular vein in the eleventh month of the females' life. Heparin was added to prevent coagulation. Cell culture was set up from lymphocytes obtained after spontaneous sedimentation. Parker's medium (TC 199), enriched to 10% with autological serum, was used for the cell culture. Wellcome's phytohaemagglutinin and fazeolin (FL 7), produced by the Animal Husbandry Institute at Balice, were used as up growth and cell

division stimulators. Cell culture was continued for 72 hours, adding Colchicine (0.001 mg/cm<sup>3</sup> of culture) 1½ hours before the end of the cell cultivation. The cell suspension was subjected to the action of hypotonic fluid (0.05M KCl) and fixed in three changes of the fixative fluid, which was a mixture of methyl alcohol with glacial acetic acid, in ratio of 3:1. A drop of the fixed cell suspension was transferred to a slide and left to air-dry. The preparations were given routine staining with 5% Giemsa stain in a Sørensen buffer with pH 6.8. Observation of metaphasal plates (200 per each female) was made either directly under a microscope or from photographic prints.

### 3. RESULTS

Analysis of the metaphasal plates of female roe deer shows that the chromosome sets of these individuals consist of 70 chromosomes. Sixty eight of them are acrocentric structures of different size, and the other two large submetacentric chromosomes, which are two X sex chromosomes (Figs. 1, 2 — Plate I). In the females examined only metaphasal plates with the 70,XX chromosome set were encountered.

### 4. DISCUSSION

In roe deer the chromosome set has been defined as  $2n=70$  by Gustavsson (1965), Amrud & Nes (1966), Wurster & Benirschke (1967) and Hsu & Benirschke (1968). Somatic chromosomes in this species of animal take on the form of acrocentric structures of different size, 68 in number. The X heterochromosome is a large submetacentric chromosome, while Y sex chromosome is shaped like a small subtelo-centric chromosome. In the Siberian roe deer (*Capreolus c. pygargus* Pallas, 1771), in addition to the chromosome set typical of the roe deer there are also microchromosomes, the so-called B-chromosomes, varying in number, up to 10 in the somatic cell (Sokolov *et al.*, 1978, Zernahle, 1980). The biological importance of these microchromosomes has not as yet been determined.

On account of the occurrence of a distinct difference between the morphology of sex chromosomes X and Y, which in the roe deer is a small and subtelo-centric structure, it would be easy to diagnose a state of lymphocytic XX/XY chimerism in individuals of the species examined. In addition to typical metaphasal plates of the female individual (70,XX) cells would appear containing XY chromosomes derived from the male partner from the period of intrauterine development, if formation of vascular connections in the placentas had taken place between them. The phenomenon of chimerism of XX/XY chromosomes is easy to trace, e.g. in the case of domestic cattle. It was even assumed that there may be a correlation between the percentage — of cells of the male co-twin and the degree of changes caused in the development of the genital organs of heifers. It turned out that there are no grounds for this suggestion (Vigier *et al.*, 1972, Marcum *et al.*, 1975). Blood cells do not take part in controlling development of the reproductive system, hence there is no simple correlation between the percentage of XY cells and deformation of the female's reproductive system. The genetic constitution plays the chief part and is expressed by

immunological-tissue reaction and hormonal effects (Jost *et al.*, 1973b, Ohno, 1979).

The failure to discover XY cells in the females examined does not completely rule out the possibility of active contact between the placentas and consequent reciprocal reaction of the embryos on each other. This might have been caused by a very weak invasion of cells of the haemopoietic tissue of the male, which in relatively small number might migrate between the foetuses and have been omitted in the study material. The lack of perceptible XY cells does not completely exclude vascular contact, migration of connective tissue cells and hormonal reaction between foetuses.

Borg's (1978) and Strandgaard's (1972b) data and the present authors' unpublished observations indicate that in about 50% of twin and multiple pregnancies individuals of unlike sex are born. If then in female roe deer originating from twin or multiple pregnancies, the partner of which during the embryonic period was a male, the phenomenon of freemartinism would be general, the proportion of unfertilized females in the population would be far higher than that found by Kałuziński (1982), i.e. only 6.5% of unfertilized females, whereas in the case of occurrence of freemartinism it could have been expected that at least 35–40% of all female roe deer in the population would be completely unfertilized. Strandgaard (1972a) observed a slightly smaller percentage of unfertilized females in the population than that found by Kałuziński (1982).

All the foregoing therefore indicates that the interplacental barrier in roe deer is most probably more hermetic than in other species with placentas of a similar type, such as domestic cattle, sheep or goats.

On the basis of analysis of chromosomes from females originating from triplet pregnancies of unlike sex and observations of the fertility of roe deer in populations, it may be assumed that there is little likelihood of the occurrence in this species of infertility etiologically connected with twin pregnancies of unlike sex, and correspond in character to the etiology of the freemartinism syndrome in cattle.

Observations up to the present indicate that in the roe deer, presumably by means of evolution, certain biological mechanisms have formed which encourage simultaneous ovulation of several egg cells, limiting early elimination of additional embryos and governing formation of barriers isolating foetal membranes of neighbouring embryos, all with the purpose of favouring attainment of higher fertility in the population.

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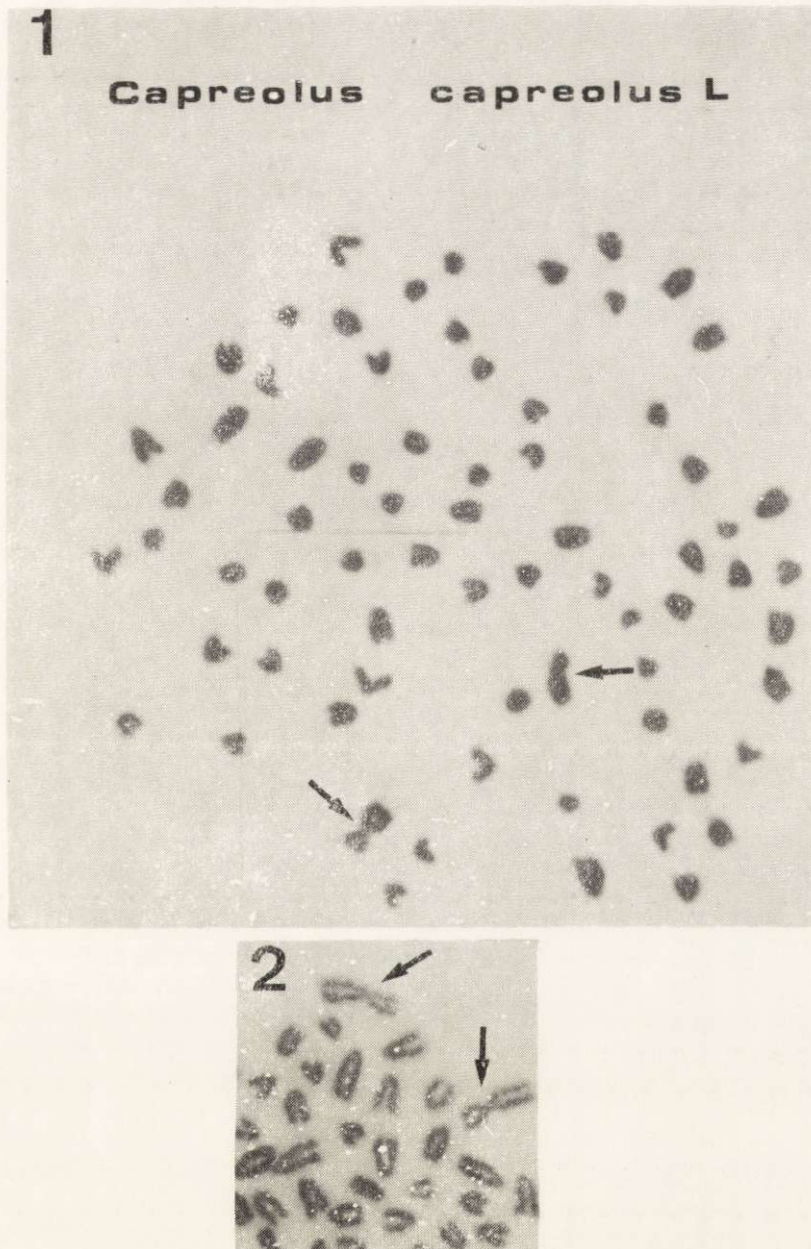


Plate I

Fig. 1. Metaphasal plate of a female roe deer born as one of triplets (♀♀♂). 70,XX karyotype. The X sex chromosomes are indicated by arrows.

Fig. 2. Part of a metaphasal plate of a female roe deer born as one of a pluriparous pregnancy (♂♂♀). The X sex chromosomes are indicated by arrows.

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## Food of the Long Eared Hedgehog in Ravines Near Agra

POKARM JEŻA USZATEGO W OKOLICACH AGRY

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Maheshwari U. K., 1983: Food of the long eared hedgehog in ravines near Agra. Acta theriol., 29, 10: 137—140 [With 2 Tables]

Annual and seasonal food composition of 165 long eared hedgehog *Hemiechinus auritus collaris* (Gray, 1830) studied from their stomach contents revealed that the main diet composed of insects (47.4% by number method and 39.74% by weight method). Beetles constituted more than 75% of the insect food. Oligochaetes, amphibians, reptiles and mammals also occurred in low proportion (7—10%). It appears that the food of the hedgehogs is related to the availability of the prey.

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### I. INTRODUCTION

The long eared hedgehog *Hemiechinus auritus collaris* (Gray, 1830) is fairly common in chalesar ravines near Agra, India. It has been

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