Phenotypic traits of skeletal anomalies observed in inbred rabbits

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SUMMARY

Inbreeding can lead to phenotypic appearance of recessive mutations, manifested via various anomalies. The purpose of the study was to evaluate the observed anomalies and the effects of narrow inbreeding in California rabbits and heterogeneous crosses. The studies were carried out in 94 sexually mature animals with inbreeding levels of Fx: 0; Fx: 0.125; Fx: 0.250; Fx: 0.375; Fx: 0.500 and Fx: 0.625 and radiographs of hip, stifle and shoulder joints, thoracic cage bones, vertebrae, maxilla and mandible were performed on 5-6 months old bunnies. Inbreeding caused mainly skeletal anomalies (limbs, vertebrae, and skeleton in general) in rabbits and the frequency of bone alterations was markedly higher in inbred heterogeneous rabbits than in inbred homogeneous ones, probably because of a lower level of selection in small farms and the maintenance of recessive genes in the genetic background of rabbit populations.

Keywords: Rabbits, inbreeding, skeleton anomalies, heterozygosis, homozygosis.

RéSUMÉ

Caractéristiques phénotypiques des anomalies du squelette observées chez des lapins consanguins

La consanguinité peut conduire à l’apparition phénotypique de mutations récessives qui se manifestent par diverses anomalies. L’objectif de ce travail était d’analyser les anomalies engendrées et les effets de l’accouplement de parents proches homogènes (race de Californie) ou hétérogènes (lapins de fermes de race indéterminée). Cette étude a été conduite sur 94 sujets sexuellement matures ayant des niveaux de consanguinité de Fx : 0, Fx : 0.125, Fx : 0.250, Fx : 0.375, Fx : 0.500 et Fx : 0.625 et des radiographies des articulations de la hanche, du genou, de l’épaule, de la cage thoracique, des vertèbres, du maxillaire et de la mandibule ont été réalisées sur les lapereaux de 5 à 6 mois. La consanguinité a induit l’apparition d’anomalies essentiellement du squelette (membres, vertèbres) et la fréquence des altérations osseuses a été nettement plus élevée chez les lapins consanguins de race indéterminée que chez les lapins homogènes de race californienne, probablement en raison d’un faible niveau de sélection dans les petites fermes et du maintien de certains gènes récessifs dans le fond génétique des populations de lapins.

Mots clés : Lapins, consanguinité, anomalies du squelette, hétérozygotie, homozygotie.

Introduction

Both the spontaneous inbreeding in natural populations and the intentional inbreeding in animal husbandry practices are prerequisites for phenotypic appearance of recessive mutations. In general, inbreeding increases the probability for obtaining homozygous recessive genotypes and that is why this breeding technique is used for detection of heterozygous individuals, carriers of unwanted recessive genes [3, 8, 14, 15].

In the domestic rabbit (Oryctolagus cuniculus), a number of abnormalities, defects and hereditary diseases are described. By means of inbred groups and inbred rabbit strains, investigations on congenital deviations related to hypercholesterolemia [1, 9, 11], primary biliary cirrhosis [13], immunoglobulinopathies [10], diabetes mellitus [7, 12, 16, 17], haematological abnormalities, various neoplastic diseases etc... have been carried out. The inbreeding results in serious abnormalities of the skeleton and the skull [6]. The autosomal recessive inheritance is responsible for the megacolon syndrome in rabbits, characterized by progressive damage of small intestines [5]. According to the most cited authors, one of the causes for the manifestation of congenital abnormalities, defects and diseases, was the inbreeding and the subsequent homozygosis of individuals of the respective population.

Data referring to the susceptibility of inbred animals to the influence of various environmental factors that, on their part, determine the appearance of some non-congenital deviations and phenocopies in the offspring are relatively few.

The purpose of the present investigations was to evaluate the observed anomalies and the effects of narrow inbreeding in California rabbits and heterogeneous crosses by means of experimental breeding schedules.

Materials and Methods

EXPERIMENTAL ANIMALS

The study on reproductive traits of female rabbits was carried out in 94 sexually mature, 7-30 months old animals. They were divided into groups as followed:
- For the heterogeneous crosses: 8 rabbits with Fx (coefficient of inbreeding): 0; 7 rabbits with Fx: 0.125; 8 rabbits with Fx: 0.250; 8 rabbits with Fx: 0.375; 8 rabbits with Fx : 0.500 and 8 rabbits with Fx: 0.625.
- For the homogeneous purebred (California): 8 rabbits with Fx: 0 ; 8 rabbits with Fx: 0.125; 8 rabbits with Fx: 0.250; 8 rabbits with Fx: 0.375; 8 rabbits with Fx: 0.500 and 7 rabbits with Fx: 0.625.

All animals in origin (breed) groups as well as in subgroups were equalized by age, with differences of no more than 4 months. Three to five litters of every rabbit doe were followed out. The number of observed bunnies in heterogeneous and homogeneous groups was uniform: 189. A total of 378 bunnies were observed. The number of controlled bunnies in heterogeneous groups was 1339, in homogeneous - 1501, i.e. a total of 2840.

RADIOGRAPHY

Radiographies were performed on 5-6 months old rabbits. The hip joints, stifle joints, shoulder joints, thoracic cage bones, vertebrae, the maxilla and the mandible were radiographed in different views. A stationary X-ray equipment TUR-800 was used. Hip joints were radiographed in ventrodorsal view, stifle and shoulder joints – in lateral views. The thorax, including the thoracic cage bones and vertebrae, were radiographed in lateral view. AGFA radiographic films, placed into metal cassettes with intensifying screens were used. Exposure was set at 10 mAs, the anodic tension - at 60 kV, and the film-focus distance - at 100 cm. The obtained radiographs were visually interpreted.

STATISTICAL ANALYSIS

Data were analysed using the fixed effect MANOVA model (Program STATISTICA, StatSoft, Inc., USA) according to the following formula:

\[ y_{ij} = \mu + \alpha_i + \epsilon_{ij} \]

where \( y_{ij} \) is the observation value of the investigated trait, \( \mu \) the population mean, \( \alpha_i \) the breed effect and \( \epsilon_{ij} \) the random errors. Differences were considered as significant when \( P \) values were less than 0.05.

### Results

Almost all observed deviations were related to skeletal abnormalities, most commonly of limbs, the vertebral column and the skeletomuscular system in general. In some cases (3.8% in heterogeneous and 0.53% in homogeneous), these anomalies affected only a forelimb or a hindlimb (figures 1 and 2), but in 1.12% in heterogeneous and 0.13% in homogeneous inbred rabbits, other bones (vertebral column, cranium) were also involved. In some animals (0.67%) mainly after weaning, two or more bone disorders were simultaneously observed (figure 3).

The Table I summarized the mean frequencies (by litter of live-born rabbits) of anomalies and disorders during inbreeding in heterogeneous and homogeneous (California) inbred rabbits. The heterogeneous inbred rabbits showed a statistically significantly higher incidence of skeletal anomalies compared to homogeneous inbred animals (\( P < 0.05 \)). In homogeneous rabbits, the only distinctive abnormality was present in the groups with Fx: 0.25 and Fx: 0.125 at a lesser extend and consisted mainly in microtia, i.e. a significant shortening of ears just above the ear pinna in one rabbit (figure 4). However, the bunny was culled when it was 50 days old and no radiography can be performed.

<table>
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<tr>
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<td>Fx: 0.625</td>
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Table 1: Mean frequencies of anomalies and disorders calculated by litter of live-born bunnies in heterogeneous and homogeneous inbred rabbits.

These observations were categorically confirmed by radiographs taken on hip (figure 5) and shoulder joints (figure 6). In a normal rabbit (figure 5A), the hip conformation presented a rectangular shape, with the deep, well C-shaped acetabulum.

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**FIGURE 1**: Forelimb abnormality (arrow) in a heterogeneous inbred rabbit with Fx: 0.250.

**FIGURE 2**: Hindlimb abnormality (arrow) in a heterogeneous inbred rabbit with Fx: 0.375.

**FIGURE 3**: Multiple complex skeletal and muscular anomalies (arrows) in a heterogeneous inbred rabbit with Fx: 0.500.

**FIGURE 4**: Homogeneous inbred rabbit (Fx : 0.250) with microtia, i.e. considerable shortening of ears over the pinna.
The femoral head is round, with smooth borders, except for the area of fovea capitis, where it is slightly flattened. The contour of the femoral head is parallel to the cranial acetabular rim up to fovea of the femoral head. The joint space is well shaped, with a uniform width. The femoral head is almost entirely fit into the acetabulum. By contrast, in an inbred heterogeneous rabbit, some marked alterations were evidenced (figure 3B): the shadow of the compact bone was less dense and thinner. In the cancellous bone of the ilium, there were osteosclerotic, osteolytic, osteoporotic and degenerative changes, revealing considerable disturbance in bone metabolism. Furthermore, the configuration between the femoral head and the acetabulum was impaired: the joint space was wider, and on the right, the femoral head was subluxated and also exhibited osteolytic, osteodystrophic and osteoporotic changes. In some cases, the sacroiliac joint was not outlined and radiological signs of arthritis in the coxofemoral joint were observed. Degenerative lesions were intense and affected the bone metaphyses: at the base of the trochanter, an elliptical shadow entirely surrounded by a sclerotic margin was sometimes observed, indicating a process of osteonecrosis. Valgus deformities (outward angulations of metaphyseal cancellous bone) were also seen. Degenerative lesions were intense and affected the bone metaphyses: at the base of the trochanter, an elliptical shadow entirely surrounded by a sclerotic margin was sometimes observed, indicating a process of osteonecrosis. Valgus deformities (outward angulations of femurs in respect to the body axis) were also seen.

As far as the shoulder joint was concerned, in a normal rabbit (figure 6A) the shadow of the head of the humerus and fossa subcapularis was well visible, and cranially, the shape of tuberositas supraarticularis was very well visualized. In front of the supraarticular thickened border, along with the scapula, a strong 3-cm long rod-shaped shadow - the shadow of the clavicle, is visible. The humerus has a shadow of a normal density, with a well defined first-order macrostructure. Serious alterations in the structure of the joint were recorded in heterogeneous inbred rabbits (figure 6B): the shadow of the fossa subcapularis was almost absent and the joint surface was markedly effaced. Over the joint surface or on the anterior rim of tuberositas supraarticularis, elliptical osteolytic foci with sharp margins were evidenced. Three lines with osteosclerotic changes were seen on the humeral head. Macrostructural alterations were also observed on the proximal humerus: destruction and disorganization of bone trabeculae of metaphyseal cancellous bone. The compact bone was thinned, with a less dense shadow, and the cortica is was lacking. The structure of the radius also exhibited characteristic alterations of osteoporosis: the medullar canal of the diaphysis was widened whereas the distal metaphysis was thickened with two clearly seen osteolytic foci.

**Discussion**

The inbreeding induced disorders observed in rabbits in the present study were essentially skeletal anomalies and were highly evidenced in inbred heterogeneous rabbits with Fx above 0.250 compared to the inbred homogenous rabbits in which the only observed alteration was microtia. This rare anomaly occurred in inbred homogenous bunnies with Fx: 0.250 and has been also described in sheep, goats, cattle and swine by VIZNER and VILLER [15]. According to the view of authors, this disorder had a different genetic background in the different species, with lethal or semi-lethal issues. Forelimbs and hindlimbs were mainly affected and bone alterations involved only one segment in the majority of cases although some multiple complex skeletal alterations were recorded in some rabbits particularly after weaning. Because these changes were mainly detected in weaned rabbits, from an ontogenical point of view, their development has begun probably earlier and thus, did not permit their detection. Furthermore, the skeletal anomalies were confirmed by radiography, the most characteristic signs being severe deformities, osteolytic, osteoporotic and osteosclerotic signs, related to joint subluxations.

The anomalies, observed in the present study, did not differ considerably from those reported by CHAI [6]. He also reported a complex of bone and skull disorders seen on radiographs in rabbits, obtained by breeding full sibs for 9-18 inbred generations: vertebral deformities, abnormalities of thoracic bone, ribs and skull - hydrocephaly, shortening or lack of the mandible etc...

Metabolic bone disorders, observed in studied rabbits, are generalized and are influenced by a number of humoral factors.
The impaired bone metabolism can result from impaired bone remodelling, in growing or adult subjects. In our study, the animals were relatively young. The metabolic alterations are known to depend on bone matrix mineralization quality. Here, an impaired bone formation and bone resorption rates with uncoupling of the former from the latter, were present. Bone disorders are influenced by the changes in the composition of circulating blood, i.e. by each metabolic abnormality. In general, the metabolic changes should be considerable prior to be reflected in the bones and joints. Many of these changes are affecting two or multiple bones at a time. They are accompanied by reduced amount of calcium salts and increase in osteoid tissue. The bone architectonics, responsible for the maximum mechanical resistance of bones, becomes impaired.

The narrow inbreeding results in a number of anomalies, but the commonest are those of the vertebral column, the limbs and the skeleton in general. The inbreeding depression has a profound negative effect on the formation of limbs, the vertebral column, the skull and other organs and systems. Many authors consider that most skeletal anomalies in mammals are dependent on recessive autosomal genes [1, 2, 4]. On the other hand, not all observed deformities are inherited. There is a possibility for the occurrence of an anomaly that is not genetically determined, but is fairly similar to the respective congenital disorder. These are the so-called phenocopies [15, 18]. According to the current study, the inbreeding in rabbits creates prerequisites mainly for the appearance of skeletal deformities. Another interesting fact is that the prevalence of observed bone and joint disorders in heterogeneous inbred rabbits was statistically significantly higher compared to that in homogeneous inbred groups. This could be explained with the lower level of selection in smaller rabbit farms, where the heterogeneous rabbits used in this study originated from. In the genetic fund of such small populations with a low level of selection, recessive genes determining a number of abnormalities are maintained for a long time [8, 14].

On the basis of our data, it should be stated that the continuous inbreeding in rabbits could be regarded as a process of rearrangement of genes in a homozygous state in a specific harmonical equilibrium. During that process, the genes, that at a given genetic background results in markedly negative effects (anomalies, product of mutational load), are manifested in the population in the course of inbreeding. These effects, due mostly to increased homozygosis and higher frequency of harmful recessives, are a classical explanation of inbred depression. Thus, as higher inbreeding level is used, as more negative cumulative effects are observed [6]. In sexually reproducing organisms, as a result of evolution, heterozygous combinations of genes that determine the heterosis effect, are much more than those, determining a specific negative mutational burden. In natural populations, these heterogeneous individuals are beneficial for the population in general, because they constantly maintain a certain level of heterozygosis. In the course of inbreeding, a continuous revision of genomes is occurring. This revision is determined by previously fixed loci and the effect of environment. Namely, this determined the effect of a given gene and genotype and the ability of an organism to become adapted to certain varying conditions or to be eliminated by the multiple environmental negative effects. That way, the progressive inbreeding leads to a gradual selection of homozygotes in respect to various loci until they all become fixed and fitted within the range of certain inbreeding minimum. The occurrence of genotype combinations with inadequately good or with bad adaptive potential, results in various phenotypic manifestations such as reduced viability, abnormalities, partial or complete sterility, and in productive animals - to reduced productivity.

References