THE SEX-DETECTION IN NEWBORN RABBITS BY *X*-CHROMATIN AND PCR-*SRY*

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ABSTRACT

The aim of the present study was gonosomal screening of newborn rabbits. The neutrophil leucocyte peripheral blood *X*-chromatins and *SRY* sequences were analysed in 16 newborn rabbits, native to two litters of female lines P91 and M91. The neutrophile *X*-chromatin was detected in 11 newborn rabbits, while *X*-chromatin was not detected in 5 young. *SRY*-PCR fragments (242 bp) were analysed in 5 newborn rabbits, while PCR fragments were missing in 11 young. All young were evaluated according to outer genitals at 3 weeks of age. By these 3 techniques were identically detected 5 males and 11 females. The appearance of the female *X*-chromatin frequency was within the range 6.66%-20%. Ranking of the neutrophile *X*-chromatin is exact, quick and relatively cheap detection system of the sex in newborn rabbits selected for tissue cultures. The authors emphasize the relevance of the cytological analysis (occurrence *X* chromatin in female neutrophils) as an additional screening for all *SRY* negative samples. This technology provides partly selective tool for categorization of the females and males in further reproductive process. Both sexes of newborn rabbits can be used for control of production of special enzymes under genetic control of the gonosomes.

Key words: Newborn rabbits, Sex-detection, Neutrophil, *X*-chromatin, PCR-*SRY*.

INTRODUCTION

The sex-detected newborn rabbits is possible to use for observation and application of active enzymes and proteins synthetized under the genetic control of gonosomes. Only two methodical approaches fulfill these demanding conditions of exactness in sex-detection of mammals at present.

Molecular PCR (polymerase chain reaction) technique for detection of *SRY* (specific region of Y chromosome) sequences is used to detect male sex and classification of inactivated *X* chromosome - *X*-chromatin, so called "drumstick" on neutrophil leucocytes of peripheral blood in females is used to detect female sex.

Briggs and Kupperman (1956), Carpentier *et al.* (1957) and Castro (1963) were the first engaged into the study of *X*-chromatin. Similar discovery made also further authors (Sun and Rakoff, 1956; Ishizu, 1993; Hochstenbach *et al.*, 1986; Sanches and Wangh, 1999; Travis, 2000; Bartova *et al*., 2001). Sexual differentiation of gonads during the development of mammals depends on presence of Y chromosome (Jost *et al.*, 1973). Testes determining gene is situated on Y chromosome. *SRY* gen is located and preserved during evolution in a certain area of Y chromosome in a broad spectrum of mammals (Sinclair *et al*., 1990). We derived the sequence of primers for amplification of specific fragment of rabbit's *SRY* gene from the sequence published in data basis of GenBank (AF230075) (Wallner *et al.*, 2001).

However, *SRY* detection is more expensive compared with the classification of neutrophils from blood smears. For these reasons we proceeded to test the suitability of neutrophils in newborn rabbits for quickly and precise sex-detection.

MATERIALS AND METHODS

Animals and experimental design

We analysed 16 one day old young rabbits in two litters of female lines P91 - cage C15: 8 young, and M91 - cage C28: 8 young on the Farm for Broiler Rabbits at the Department of Small Farm Animals, SARC Nitra. We marked the young by cut into ear flaps after a beforehand settled key. At the age of three weeks we detected the sex in each rabbit by visual detection of outer sexual organs.

Cytological Analyses

We used the blood from cuts into ear flaps for blood smears and classification of neutrophils. We fixed the blood smears by Cytofix after drying, and the dried preparations were stained by 2% Giem's dye for 15 minutes. We evaluated 30 neutrophils in each one day old rabbit (totally 480) for the presence (females) or absence (males) of *X* chromatin -"drumstick"-inactivated *X* chromosome (Figures 1 and 2).

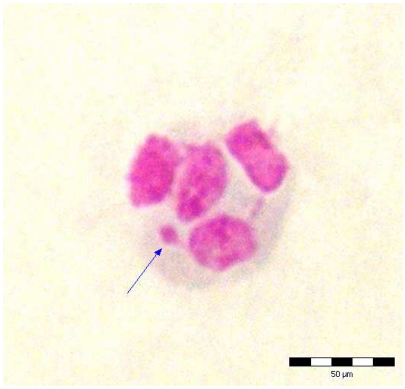

Figure 1: Neutrophil of female (*XX*) *X* chromatin (marked by arrow)

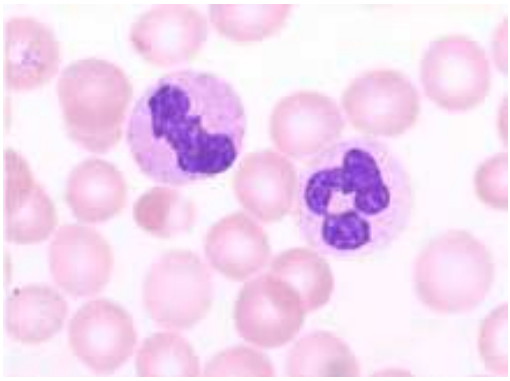

Figure 2: Neutrophils of male (*X*Y) without *X* chromatin

Molecular Analyses

We obtained samples of DNA isolated from pieces of skin tissue, cut for identification of individual young, to detect *SRY*. About 200 ng DNA were used for PCR. Amplification of *SRY* fragment was done in 35 cycles under following conditions: first denaturation step 94°C 2 min, 94°C 20 sec, 64°C 30 sec, 72°C 30 sec, last step 72°C 10 min) using specific primers Oc*SRY*21F: 5' - AGC GGC CAG GAA CGG GTC AAG - 3', and Oc*SRY*23R: 5' - CCT TCC GGC GAG GTC TGT ACT TG - 3'. Obtained PCR products were analysed by electrophoresis in 3% agarose gel containing ethidium bromide and visualized by video-documenting system GDS8000 (Figure 3).

RESULTS AND DISCUSSION

During identification of neutrophils in one day old young of female P91-C15 we identified 5 young with *X* chromatin (Figure 1) and 3 young without *X* chromatin (Figure 2). Female M91- C28 had 6 young with *X* chromatin on neutrophils and 2 young without the presence of *X* chromatin in the litter. *SRY* analyses gave data for molecular identification of sex in one day old young of rabbits on the basis of detected PCR fragments in males (Figure 3, line *M* - marker of molecular weight 50 bp DNA Step Ladder Promega, Cat.no. G4521, lines 2, 5, 7, 13, 14, line No. 15 positive control). Size of PCR products in males is 242 bp. Fragments are not present in females (lines 1, 3, 4, 6, 8-12). In the litter of female P91-C15 were detected 3 males on the presence of *SRY* gene, absence of *SRY* was confirmed in 5 females. In the litter of female M91-C28 were identified 2 males and 6 females.

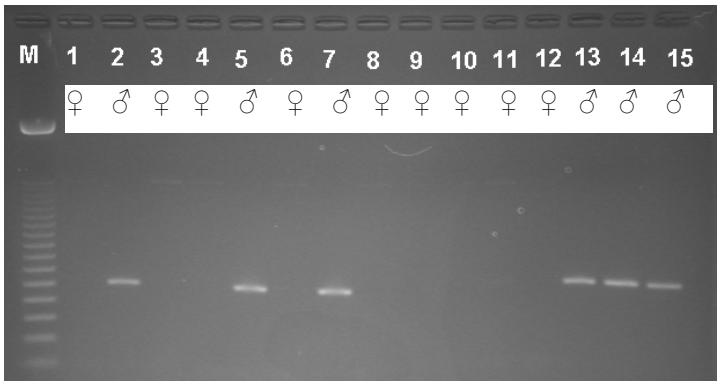

Figure 3: Representative results PCR - *SRY* (PCR-polymerase chain reaction. *SRY*–specific region of Y chromosome. PCR products in males have size 242 bp. Fragments are not present in females)

In three weeks of age we verified the results obtained from detection of *X* chromatin on neutrophils of peripheral blood, from *SRY* analyses and sex-detection according to outer sexual organs in individual young (Table 1).

We used the results of *SRY* analyses, occurrence of *X* chromatin on neutrophils and identification key represented by cuts in ear flaps of young and we confirmed unambiguously the accuracy of used techniques and methods. Female P91-C15 had 5 females in the litter, their identification cut was: 1-3-4-6-8, and 3 males with identification cut: 2-5-7. Female M91-C28 had 6 females in the litter, with identification cut: 1-2-3-4-7-8, and 2 males: 5-6. Absence of *SRY* sequence corresponds with the presence of X chromatin on neutrophils in identified one day old females. Indeed, false negative results may occur for *SRY* detection. Therefore, we would like to emphasize the relevance of the cytological analysis (occurrence *X* chromatin in female neutrophils) as an additional screening for all *SRY* negative samples.

Table 1: Sex-screening of young rabbits

Characteristics		Youngs of M91 line	Youngs of P91 line	Result of analysed young rabbits
SRY	+	2	3	5
SRY	-	6	5	11
Presence of neutrophils without X- chromatin	-	2	3	5
Presence of neutrophils with X- chromatin	+	6	5	11
Visual sex-detection of youngs at the age 3 weeks	♂	2	3	5
	♀	6	5	11

SRY – specific region of Y chromosome; Neutrophils without *X*- chromatin (No=384–448; 80-94% from total amount evaluated neutrophils = 480; 30 neutrophils from each animal); Neutrophils with *X*-chromatin (No=32-96; 6,66-20% from total amount evaluated neutrophils=480; 30 neutrophils from each animal)

Classification of 480 neutrophils from 16 rabbit young - 30 neutrophils from each animal - showed the occurrence of *X* chromatin frequency in female sex to be from 6.66% to 20%. Mentioned findings correspond with identification of 2 - 6 *X* chromatins per 30 analysed neutrophils from one female. We can state that sex detection in one day old young on the basis of identification of *X* chromatin presence on neutrophils of females is a quick, economically not demanding and suitable method for selection of male individuals intended for utilization in techniques of tissue cultures. One day old females of identified sex are given back to their mothers. This selection intervention provides unimpaired continuity of reproduction process in the given experimental herd of rabbits. Selection of one day old males saves housing and feed.

One day old rabbits detected in such way are prepared for further genetic or breeding purposes. At need it is possible also to use tissue cultures of both sexes of young for production of special biologically active substances synthetized under control of sexual chromozomes *X* or *Y*. Besides, the application of xenografts depends also on sex of donor and recipient.

CONCLUSIONS

Sixteen one day old rabbits, from two litters of P91 and M91 female lines, were analysed for the presence of *X* chromatin of neutrophil leucocytes of peripheral blood and *SRY* sequence. *X*-chromatin on neutrophils was detected in 11 newborn rabbits, whereas the *X*-chromatin was absent in 5 young. By *SRY* analyses were classified PCR fragments (242 bp) in 5 young, whereas PCR fragment did not occur in 11 young. Analysed young were examined according to outer sexual organs at the age of 3 weeks. By these 3 techniques were concurrently detected 5 males and 11 females. Frequency of *X* chromatin occurrence in female sex was within the span 6.66-20%. Classification of *X*-chromatin of neutrophils is exact, quick and quite cheap method of sex detection in newborn rabbits selected for tissue cultures. This technique provides selection tool for incorporation of females into further reproduction process. The methods gives possibility of special utilization of newborn rabbits of both sexes to observe the production and application of active enzymes and proteins synthetized under genetic control of gonozomes. Besides this, the application of xenograft depends also on sex of the donor and recipient.

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