Aneuploidy in the Transgenic Rabbit

(transgenic rabbit / lymphocyte / c-metaphase / chromosome / aneuploidy / G-banding)

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Abstract. The aim of this study was to determine whether there are differences in the karyotypes between transgenic and non-transgenic or control rabbits. New Zealand White transgenic rabbits (F1 generation) were obtained after breeding of transgenic founder rabbits that were derived from single – SM – or double microinjection – DM – with a *WAP-hFVIII* transgene. C-metaphase plates were obtained from short-time culture of peripheral blood lymphocytes synchronized by the addition of colcemide. A significantly higher rate of aneuploidy was observed in c-metaphase spreads of transgenic (56–66%) rabbits, as compared to non-transgenic ones (28–38%) ($P < 0.05$; $P < 0.01$). The patterns of chromosome banding were identical in both groups of rabbits. No structural aberrations were revealed in either group. These findings demonstrate that transgenic rabbits have a higher frequency of numerical chromosomal aberrations in their peripheral blood lymphocytes than normal rabbits, but without apparent deleterious effects on health or reproduction.

Transgenic animals represent an alternative way to produce biologically active proteins (Lubon and Paleyanda, 1997). Transgenic rabbits have become a useful model system for the study of the genome, gene expression and regulation, as well as for the production of recombinant proteins in the mammary gland (Stromqvist et al., 1997). However, the random integration of a transgene can disrupt the function or regulation of an endogenous gene, resulting in insertional mutations (Palmiter and Brinster, 1986), or chromosomal aneuploidy (Goepfert et al., 2000). Aneuploidy is the gain or loss of chromosomes as a result of meiotic and mitotic non-disjunction events, which can also lead to an alerted gene balance and loss of heterozygosity (Goepfert et al., 2000). Numeric chromosomal polymorphisms have been described in both pathological and normal conditions and are a characteristic in mammals (Jacobs et al., 1966). Zartman and Fechheimer (1967) claim that the level of aneuploid cells in mammals is genetically determined.

In rabbits, the rate of aneuploidy varies depending on the tissue of origin, from 5% in *in vitro* fertilized oocytes (Asakawa et al., 1988), 16–18% in blood cells (Zartman and Fechheimer, 1967; Parkányi, 1981), and 35% in bone marrow (Parkányi, 1981), up to 56–83% in embryos (Austin, 1967; Shi et al., 2004). Likewise, 17% of *in vitro* fertilized oocytes were polyploid, versus only 9% of *in vivo* fertilized oocytes (Asakawa et al., 1988).

Besides these numeric alterations in chromosomes, there are structural aberrations, which can be detected by using different banding techniques. R-banding and G-banding of standard rabbit karyotypes was presented by Hayes et al. (2002), in agreement with the 1981 G-banding standard nomenclature (Committee for Standardized Karyotype of *Oryctolagus cuniculus*, 1981). Although Lipinski et al. (2003) did not observe structural aberrations in WAP-hGH transgenic rabbit chromosomes by the GTG-banding pattern, we believe that the cytogenetical analysis of transgenic animals can contribute to eliminating chromosome aberrations in transgenic offspring and explain some health problems. Our present study was aimed at comparing the karyotypes of transgenic and non-transgenic rabbits by evaluating the aneuploidy rate and the pattern of chromosomal G-banding.

Material and Methods

Animals

In our experiments we used transgenic rabbits of the F1 generation. They were obtained from the breeding of both lines of transgenic founders (SM – single microinjection and DM – double microinjection with the *WAP-hFVIII* gene) with non-transgenic rabbits of the same breed (New Zealand White). Founders were produced as described by Chrenek et al. (2002).

Chromosome preparation

Venous blood samples were collected aseptically from the ear vein of transgenic and non-transgenic

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Table 1. Chromosomal analysis (c-metaphases) of transgenic and non-transgenic rabbits

Groups of rabbits	Diploidy (2n = 44)	Polyploidy (≥ 3n)	Aneuploidy		
			Hypodiploidy (2n < 44)	Hyperdiploidy (2n > 44)	Total
DM					
male 36-2	17(34%)	0 (0%)	31(62%)	2(4%)	33(66%)
female 36-1	20(40%)	2 (4%)	17(34%)	11(22%)	28(56%)
average:	19(38%) [A]				31(61%) [a]
SM					
male 1-4	21(42%)	0(0%)	26(52%)	3(6%)	29(58%)
male 1-15	17(34%)	0(0%)	33(66%)	0(0%)	33(66%)
female 1-3	18(36%)	0(0%)	31(62%)	1(2%)	32(64%)
average:	19(38%) [A]				31(63%) [a]
NORMAL:					
female 1	29(58%)	2(4%)	19(38%)	0(0%)	19(38%)
female 2	34(68%)	2(4%)	13(26%)	1(2%)	14(28%)
average:	32(63%) [B]				17(33%) [b]

[A] vs [B], and [a] vs [b] differ significantly at $P < 0.01$. NORMAL: non-transgenic; SM: single microinjection; DM: double microinjection – are transgenic groups.

rabbits, using sterile needles and plastic syringes. Whole blood (0.25 ml) was added to complete chromosome medium 1A (4 ml) (Life Technologies Ltd., Paisley, UK). The blood cultures were incubated at 37°C for 71 h. Colcemide (0.6 µg/ml) (Life Technologies) was added 90 min prior to cell harvest. After a 15 min hypotonic treatment with 0.075 M potassium chloride and fixation in modified Carnoy's solution (3 : 1, methanol : acetic acid), the resuspended cells were spread on frozen glass microslides, air-dried, and stored unstained at room temperature; or stained for 10 min with a 2% Giemsa solution, or with 0.2 µg/ml propidium iodide. Stained microslides were observed under the Leica microscope. The chromosomal analysis was carried out from chromosome microphotographs, using 50 c-metaphase spreads for numerical and 15 G-banded metaphase spreads for structural evaluation per rabbit. The G-banding pattern was obtained upon treatment with 0.025% trypsin solution, at 25°C for 15 s.

Statistics

The χ^2 test was used to compare chromosomal aneuploidy between transgenic and non-transgenic rabbits.

Results

Study of numeric aberrations

Cytogenetic analysis by G-banding of c-metaphase spreads of 5 transgenic and 2 non-transgenic rabbits was carried out. The rate of aneuploidy observed in transgenic rabbit chromosomes was significantly higher ($P < 0.05$ and $P < 0.01$, respectively) than that in non-transgenics (Table 1).

Transgenic rabbits were characterized by a higher frequency of aneuploidies (56–66%), as compared to non-transgenic rabbits (28–38%). On the other hand, non-transgenic females exhibited a higher rate of diploid somatic cells (63%) than the transgenic rabbits (38%) (Fig. 1; $P < 0.05$). Most of the aneuploidy was attributable to the category of hypodiploid cells ($2n < 44$; Fig. 2), whereas the frequency of hyperdiploid cells ($2n > 44$; Fig. 3) was extremely low. There were no differences in the rate of aneuploidy between SM- (63%) and DM-derived (61%) transgenic rabbits, Table 1. Polyploid cells (≥ 3n; Fig. 4) occurred sporadically in both groups of rabbits (0%–4%).

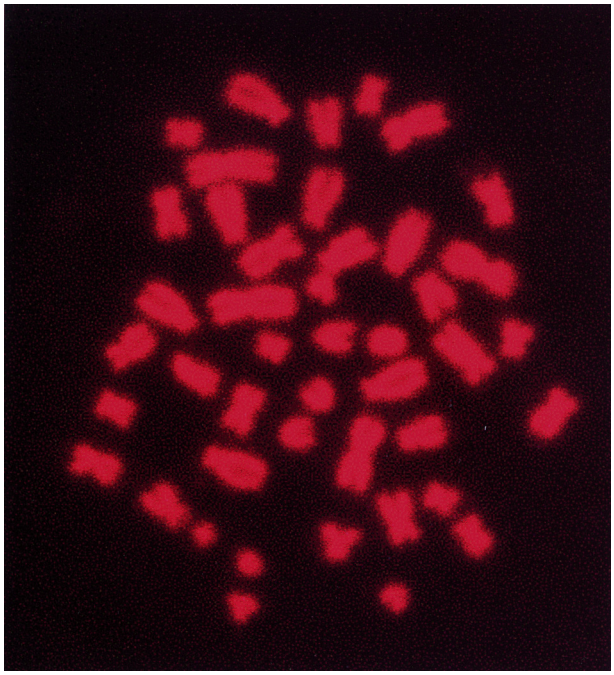

Fig. 1. Diploid c-metaphase of a male rabbit, 2n = 44, XY, stained with propidium iodide

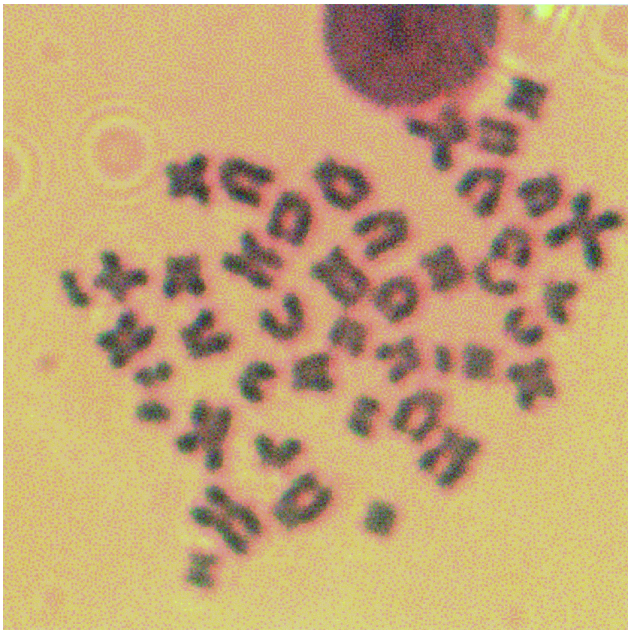

Fig. 2. Hypodiploid c-metaphase of a female rabbit, 2n = 44 - 1, XX, Giemsa staining

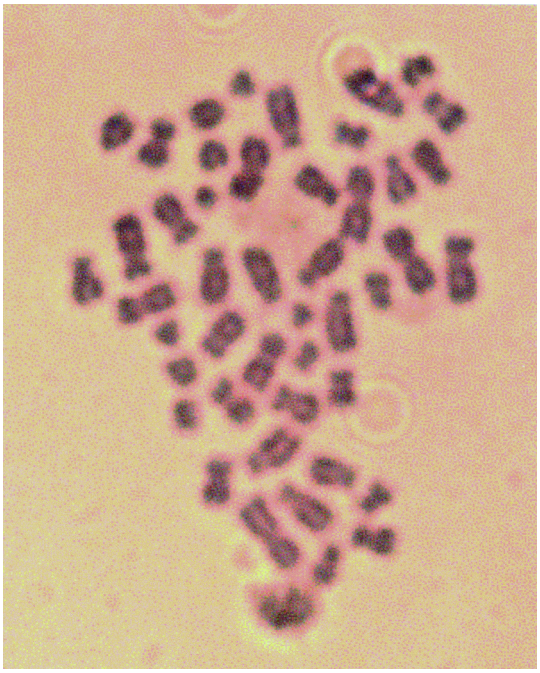

Fig. 3. Hyperdiploid c-metaphase of a female rabbit, 2n = 44 + 1, XX, Giemsa staining

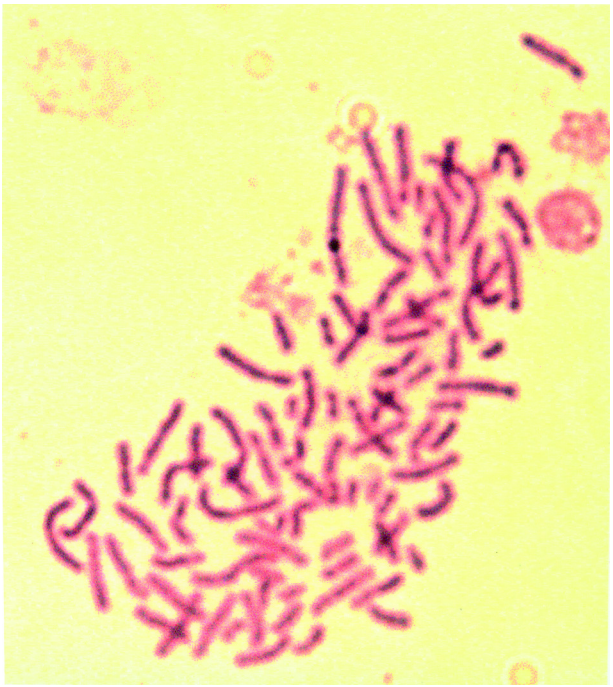

Fig. 4. Polyploid c-metaphase of a rabbit (4n), Giemsa staining

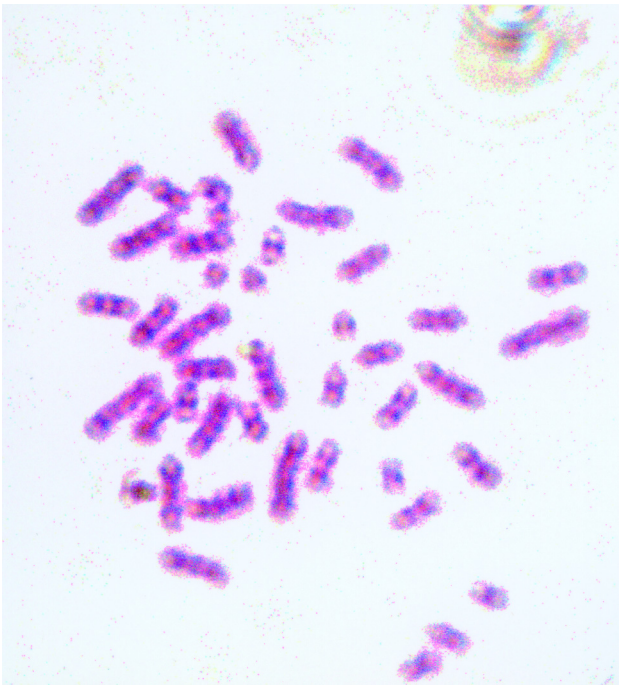

Fig. 5. G-banding c-metaphase of a non-transgenic female rabbit, 2n = 44, XX

Study of structural aberrations

We evaluated the G-banded karyotypes of our rabbit preparations using the G-banding standard nomenclature (Committee for Standardized Karyotype of *Oryctolagus cuniculus*, 1981). According to this nomenclature, G-banded rabbit karyotypes consist of four groups: A, B, C, D and sex chromosomes X, Y. We observed all 4 chromosome types: A-metacentric, B-submetacentric, C-subtelocentric, telocentric, and D-acrocentric (Figs. 7, 8). We constructed a G-banding idiogram of the rabbit chromosomes (Fig. 6). G-banding chromosome analysis revealed no structural aberrations

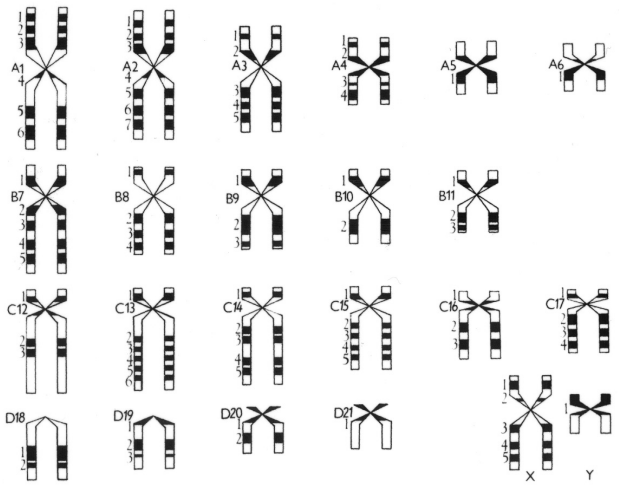

Fig. 6. G-banding idiogram of the rabbit

in transgenic or non-transgenic rabbits. The number and localization of chromosome bands was identical in both groups. No differences in G-banding patterns were revealed between the karyotypes of transgenic males (Fig. 7) and females (Fig. 8).

Discussion

Cytogenetic analyses were carried out on the peripheral blood lymphocytes of transgenic and non-transgenic rabbits of both sexes, using the technique of culture in complete chromosome medium, according to Goepfert et al. (2000) and Lipinski et al. (2003), Shi et al. (2004). We report, for the first time, numerical chromosome aberrations, or aneuploidies, in the chromosomes of transgenic and non-transgenic rabbits. Chromosomal aneuploidy can result from meiotic and mitotic non-disjunction events (Goepfert et al., 2000; Shi et al., 2004). Our results on the frequency of aneuploid cells are consistent with the reports of Zartman and Fechheimer (1967), who claimed that the rate of aneuploid cells in mammals was genetically determined. Obviously, transgenic rabbits are genetically different from non-transgenic rabbits. This is confirmed by our study, where significant differences between transgenic and non-transgenic rabbits in the frequency of chromosomal aneuploidies were revealed.

Transgenic rabbits exhibited significantly lower numbers of diploid somatic cells (34–42%) than non-transgenic rabbits (58–68%). This is probably because transgenic rabbits have a significantly higher level of mitotic non-disjunction (Goepfert et al., 2000). According to these authors, the processes of chromosome segregation and movement are well defined at the cellular level, whilst molecular events that interfere with appropriate chromosome segregation and result in aneuploidy are not well understood and are likely to involve multiple targets. For instance, it is assumed that aneuploidy is generated by numerous factors including

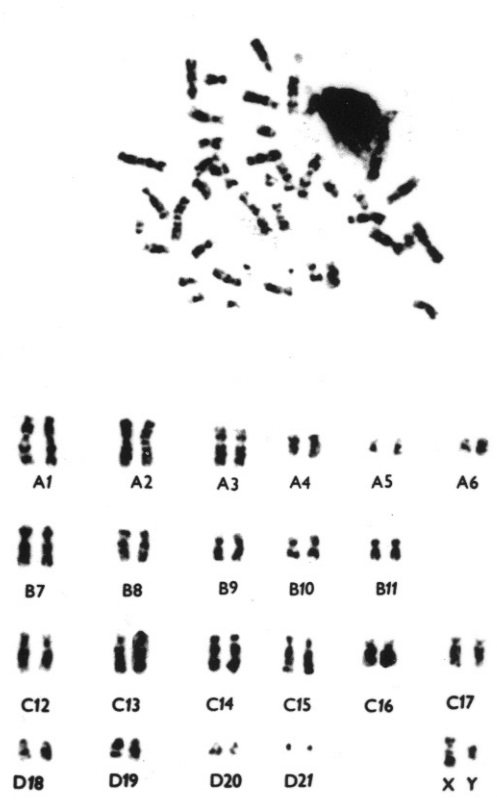

Fig. 7. G-banding karyotype of a transgenic male rabbit, 2n = 44, XY

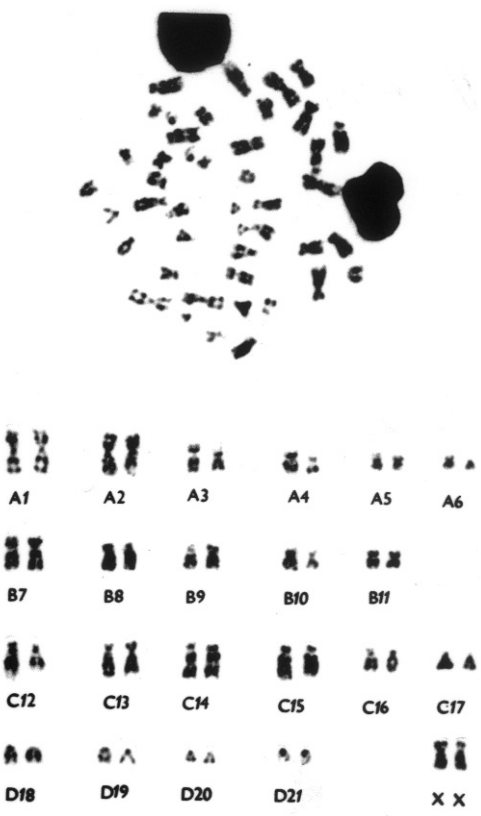

Fig. 8. G-banding karyotype of a transgenic female rabbit, 2n = 44, XX

interference with mitotic spindle dynamics, abnormal centrosomes, duplication, altered chromosome condensation and cohesion, defective centromeres, and the loss of mitotic checkpoints (Goepfert et al., 2000). However, among the aneuploid cells, the occurrence of hypodiploid cells was higher than that of hyperdiploid cells for both transgenic and normal rabbits. Polyploid cells (≥ 3n) occurred sporadically (0–4%) in both groups of rabbits.

The rate of aneuploidy in different studies also varied depending on the cell source. Thus, about 17% of cells from rabbit blood cultures were aneuploid (Zartman and Fechheimer (1967), 18% from rabbit lymphocytes, about 35% from bone marrow (Parkányi, 1981), 63% from rabbit blastocysts (Austin, 1967) and as high as 83% from cumulus cell nuclear transfer-derived rabbit embryos (Shi et al., 2004). DNA aneuploidy of single blastomeres is common in this species, and embryonic viability was not affected by the presence of aneuploid blastomeres; thus, a limited number of abnormal blastomeres were compatible with normal preimplantation development (Schumacher, 1993). However, Shi et al. (2004) demonstrated significantly increased chromosomal aneuploidy rates in cumulus cell-nuclear transfer rabbit embryos and embryos derived from nuclear transfer of rabbit fibroblasts into bovine oocytes, compared to *in vivo* fertilized rabbit embryos. The incidence of chromosomal abnormalities was correlated with subsequent developmental failure. This suggested to us that genetic manipulation of embryos may also lead to increased aneuploidy. However, in our experiments there were no differences in the aneuploidy rate between transgenics derived by single microinjection (63%), or by double microinjection (61%), suggesting that DM did not result in increased aneuploidy and can be safely used to increase transgenesis frequency.

The karyotype stability of rabbit cells *in vitro* is well known to correlate with the origin of the cell lines, as well as their age in culture (Yang, 1977). The type of medium used to grow rabbit cells has also been shown to influence their chromosomal stability (Earley, 1976). Lines derived from cornea, spleen, and kidney showed high frequencies of polyploidy in passages as early as 15, whereas lines derived from lung and skin retained the normal karyotype much longer, up to 60 passages. In later passages, the cells became pseudodiploid, hyperdiploid, or polyploid (Yang, 1977). Thus, the higher percentage of chromosomal aneuploidy in our samples (28–66%) may be explained by the greater age of our rabbits (more than 1–1.5 years) and different stabling conditions (air ventilation, the way of slurry removal) than in our earlier work (Parkányi, 1981).

Throughout the study on structural aberrations, we optimized the procedure for G-banding of c-metaphase chromosomes by choosing appropriate conditions of predigestion, using a 15 s exposure in 0.025% trypsin solution at 25°C. Our data on G-banding karyotypes and c-metaphases in both groups of rabbits are in agreement with the G-banded standarded nomenclature (Committee for Standardized Karyotype of *Oryctolagus cuniculus*, 1981). Based on the GTG-banding pattern, no structural aberrations were observed either in transgenic or non-transgenic rabbit metaphase chromosomes. Similarly, no differences in G-banding metaphases between the sexes were noted. These findings correspond with the observations of Lipinski et al. (2003), who did not detect any effect of the *hGH* transgene on chromosomal banding.

In conclusion, this is the first report of a higher rate of aneuploidy in transgenic rabbit peripheral blood cells, as compared to normal animals.

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