Acta vet. scand. 1972, *13*, 151—160.

From the Department of Animal Genetics, Nutrition and Hygiene, Royal Veterinary College, Stockholm, Sweden.

# EMBRYONIC DEATH IN PIGS CAUSED BY UNBALANCED KARYOTYPE*

By

*A. Åkesson* and *B. Henricson*

ÅKESSON, A. and B. HENRICSON: *Embryonic death in pigs caused by unbalanced karyotype.* Acta vet. scand. 1972, *13*, 151—160. — A landrace boar being heterozygous for a translocation has been used in 43 matings to 30 sows. Compared to a normal boar which also served part of the same sow group the defect boar had a by 30 % reduced fertility. Five different types of unbalanced karyotypes were detected in fetuses sired by the translocation heterozygote. Since no unbalanced karyotype was to be found postnatally in 111 offspring, all defect karyotypes seem to be lethal at the embryonic stage. Forty-one % of the postnatally investigated cases had the same translocation as the father.

embryonic death; karyotype, unbalanced; translocation; pigs.

*Henricson & Bäckström* in 1964 described a Swedish landrace boar with reduced fertility being heterozygous for a chromosomal translocation. The boar had in 21 litters produced an average number of 5.6 piglets per litter. When mated to a different boar the same sows had an average litter size of 12.7. The fertility reduction can consequently be estimated to about 56 %.

A son of the above mentioned boar, which inherited the translocation from his father, was used in a series of matings. This boar (545) also gave a reduced litter size. It has to be suspected that the fertility reduction is caused by gametes with an unbalanced karyotype, being produced by the boar and causing early embryonic death.

* The investigation was supported by the Swedish Council for Forestry and Agricultural Research.

The theory of unbalanced karyotypes being transmitted from the boar of his offspring was tested in the present investigation. The chromosome constitution of the embryonic offspring of boar 545 was investigated. Also postnatal offspring was included in the experiment.

## MATERIAL

The boar 545 produced a total of 43 litters with 30 different sows. Twelve sows (all gilts) were slaughtered in different stages of gestation and the chromosomes of 113 embryos or fetuses were investigated. Eight sows produced 14 litters with 117 piglets. These were all karyotyped during the first week of life or, in case of stillbirth, immediately after birth. The remaining 17 litters were produced by 10 sows. For these only the number of piglets born and the status of the piglets until eight weeks of age have been noticed. These piglets were used in separate experiments. One normal boar (1102) produced during the same time 592 piglets in 46 litters with 18 sows. These sows formed one part of those sired by the boar 545 in different breeding periods. Thus the two boars have given offspring alternatively with 12 sows.

## METHODS

For the chromosome analyses the following methods were used:

*Living animals.* Leucocyte culturing and preparation according to *Moorhead et al.* (1960). Skin culture according to *Basrur et al.* (1963).

*Stillborn animals and fetuses.* Culture of skin or other tissues according to *Basrur et al.*

*Embryos.* Squash preparations after preparation with colchemid and hypotonia, mainly according to *Mc Feely* (1966).

Varying numbers of metaphases were inspected from different cases depending on how well cultures and chromosome preparations succeeded. A chromosome diagnosis was based on not less than 10—15 metaphases. The number of chromosomes per metaphase varied to some extent due to preparation artefacts. Five per cent at the most deviated from the number forming the final diagnosis.

The collection of embryos was performed according to *Mc Feely* (1966).

## RESULTS

Table 1 presents the number of offspring from the translocation heterozygote (545) and the normal boar (1102). The number and frequency of stillborn piglets are also given. The boar 545 had reduced number of piglets per litter as compared to the normal boar. The reduction amounts to 32 % in the male sex and 35 % in the female sex, giving an average reduction of 34 %.

Table 1. Total offspring and stillborn offspring sired by the translocation heterozygote (545) and by a normal boar (1102).

| Boar | Number of sows | Number of litters | Number born | | | | | | Number of stillborn per litter | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | total | | | per litter | | | | | |
| | | | ♂ | ♀ | sum | ♂ | ♀ | sum | ♂ | ♀ | sum |
| 545 | 18 | 31 | 133 | 129 | 262 | 4.3 | 4.2 | 8.5 | 0.3 | 0.3 | 0.6 |
| 1102 | 18 | 46 | 291 | 301 | 592 | 6.3 | 6.5 | 12.8 | 0.6 | 0.4 | 1.0 |

The offspring of 545 held only one piglet with macroscopically detectable malformation: a case of cleft palate. Two malformed cases — one with severe umbilical hernia and one intersex — were included in the offspring of 1102. The frequency of stillborn piglets (Table 1) was lower in litters sired by 545 than by those sired by 1102. This should be a consequence of the corresponding difference in the litter size. None of these malformed or stillborn piglets had an abnormal chromosome constitution.

Chromosome analyses were carried out on 117 piglets from 14 litters sired by 545. It was possible to get a chromosome diagnosis on 111 of these. The karyotype status was the following

| Normal | | Translocation heterozygotes | |
|---|---|---|---|
| ♂ | ♀ | ♂ | ♀ |
| 28 | 37 | 24 | 22 |

No abnormal karyotypes could be detected in addition to the translocation. This appeared in 46 % of the male sex and in 37 % of the female; an average of 41 %.

The karyotypes of the abnormal boar 545 and of his "translocated" offspring were in complete morphological agreement

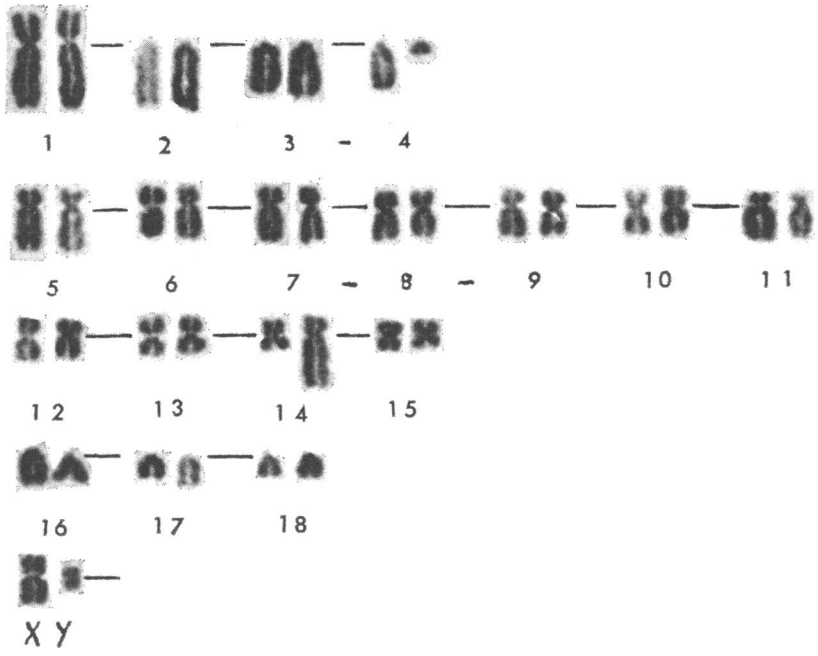



Figure 1. Karyotype of the translocation heterozygote 545.

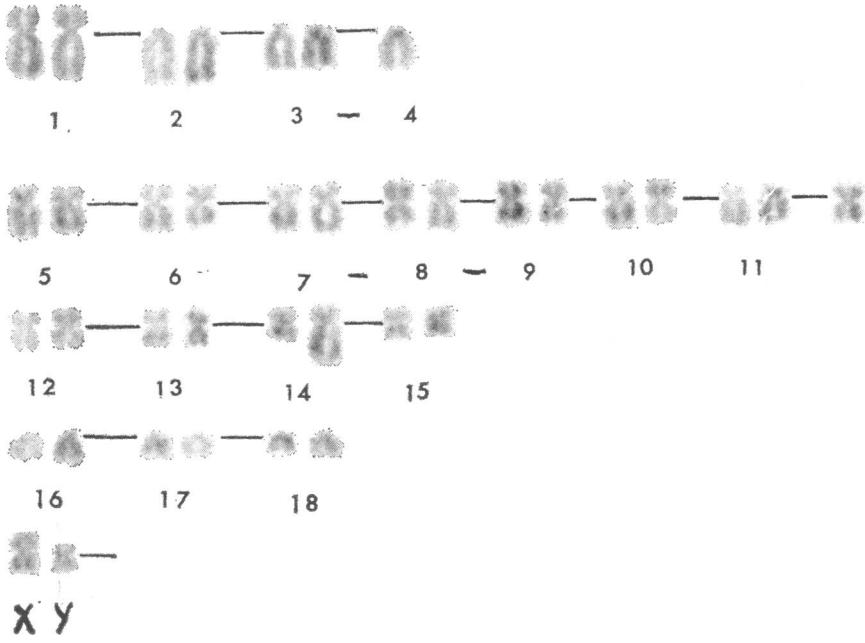



Figure 2. Unbalanced karyotypes in embryos.
38 XY (4, 14+T+(7—9)).

with that of the father of 545 as described by *Henricson & Bäckström* (1964). In Fig. 1 one such idiogram is presented. The same grouping of chromosomes was used as in the article just mentioned. Translocation had taken place between one of the autosomes of pair no. 4 and one of the pair no. 14. Presumably the translocation is reciprocal, but the morphologically detectable result is that the major part of no. 4 has attached to no. 14. These two new chromosomes will be called T (14 + the major part of no. 4) and F (the remaining part of no. 4).

In Table 2 the analysis of embryos and fetuses from 12 gilts sired by 545 is presented. Two gilts were slaughtered 10—11 days after service, seven after 22—28 days and three after 63—88. One-hundred-and-thirty-one embryos or fetuses were detected or

Table 2. The embryo status of 12 gilts served by the boar 545.

| Gilt no. | Slaughtered days after breeding | Number of corp. lut. | Number of embryos | Chromosome status | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | XY | XY+T+F | XX | XX+T+F | notes, other anomalies |
| 1 | 10 | 13 | 11 | 4 | 2 | 2 | 1 | 2 dead |
| 2 | 11 | 12 | 12 | 3 | 2 | 2 | 1 | 4 not analysed |
| 3 | 22 | 16 | 11 | 2 | 3 | 1 | 3 | 1 XY 4, 14+14+T;<br>1 XX 4, 14+14+T |
| 4 | 23 | 13 | 13 | 4 | 3 | 3 | 3 | |
| 5 | 24 | 11 | 8 | 1 | 1 | 1 | 2 | 1 XY 4+4+F, 14;<br>2 dead |
| 6 | 25 | 12 | 11 | 3 | 3 | 2 | 0 | 1 XY 4, 14+T+(7—9)<br>2 not analysed |
| 7 | 25 | 13 | 12 | 3 | 4 | 1 | 0 | 1 dead, 3 not analysed |
| 8 | 25 | 13 | 12 | 3 | 2 | 2 | 3 | 2 XY 4, 14+14+T |
| 9 | 28 | 14 | 11 | 1 | 1 | 2 | 4 | 1 XY 4+F, 14+14;<br>1 XX 4+4, 14+T;<br>1 dead |
| 10 | 63 | 12 | 8 | 2 | 3 | 0 | 1 | 1 XY 4, 14+14+T;<br>1 XX 4, 14+14+T |
| 11 | 80 | 17 | 12 | 3 | 1 | 2 | 2 | 2 XY 4, 14+14+T;<br>2 dead |
| 12 | 88 | 15 | 10 | 2 | 4 | 3 | 0 | 1 not analysed |
| Total | | 161 | 131 | 31 | 29 | 21 | 20 | |

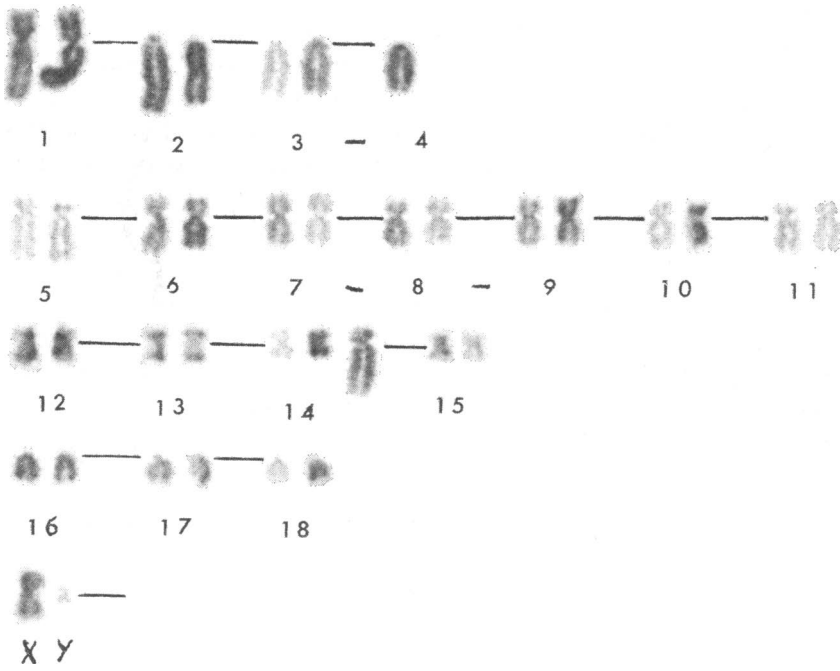



**Figure 3.** Unbalanced karyotypes in embryos. 38 XY (4, 14+14+T).

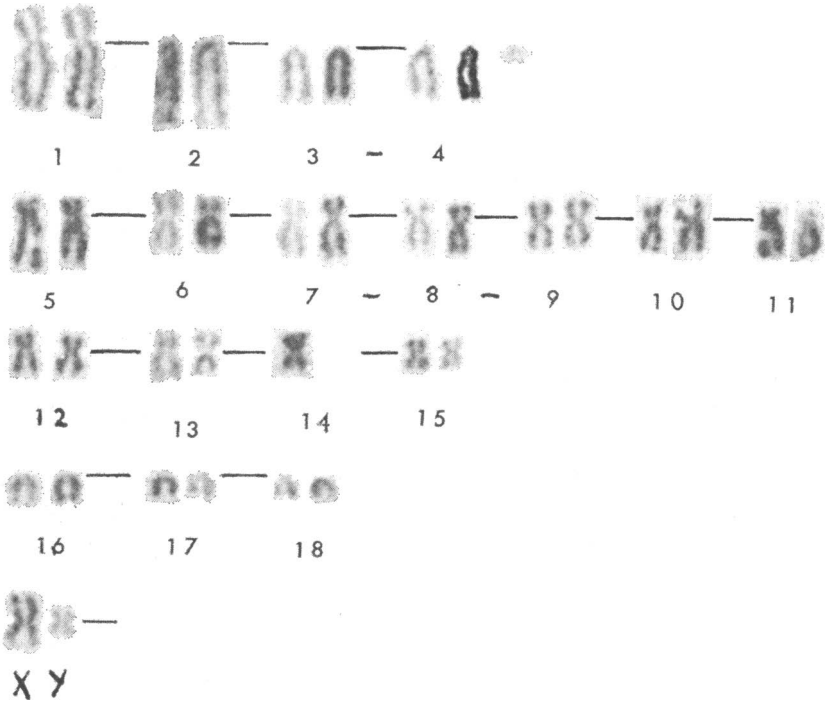



**Figure 4.** Unbalanced karyotypes in embryos. 38 XY (4+4+F, 14).

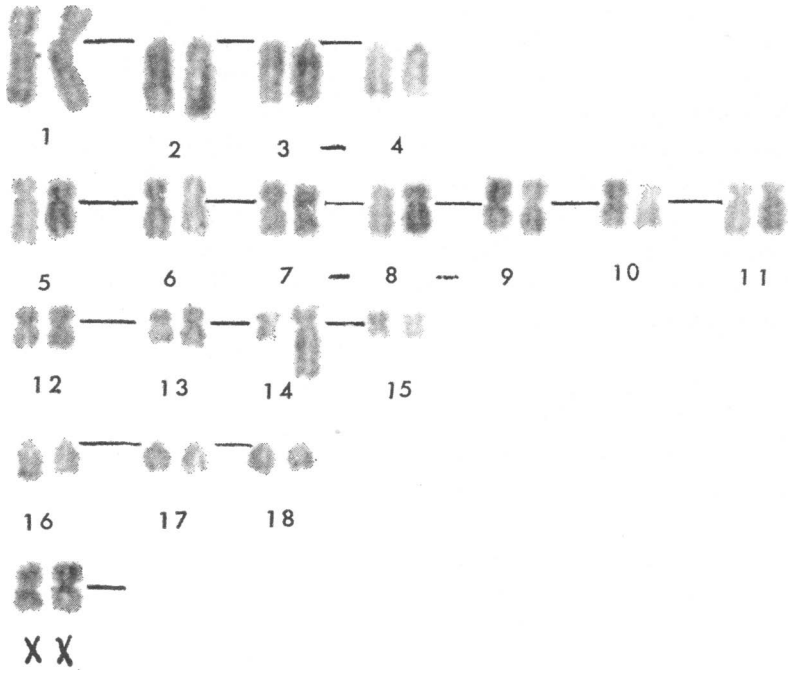
10.9 per litter. The numbers of embryos correspond to 81 % of the number of yellow bodies detected in the ovaries. Consequently 19 % of the number of ovulated ova have disappeared. Another eight embryos (5 %) were dead or the subject for different degrees of degeneration at the moment of investigation. All three age groups had dead embryos.

The chromosome analyses were successful for 113 cases, which means 91 % of the number detected. Translocation heterozygotes formed 48 % of diagnosed karyotypes of both sexes. The female piglets investigated post partum were consequently underrepresented by 11 % as compared to female embryos or fetuses. The difference was not significant, however. In addition to the translocation, five different abnormal karyotypes were detected. All included one of the elements (T or F) interested in the translocation. Four abnormities were limited to the chromosomes nos. 4 and 14. One case also interested one chromosome from the submetacentric group, nos. 7—9. All karyotypes consisted of 38 chromosome elements. The unbalanced karyotypes were distributed in the following way. The karyotype of the translocation heterozygote has the following representation: (4 + F), (14 + T).

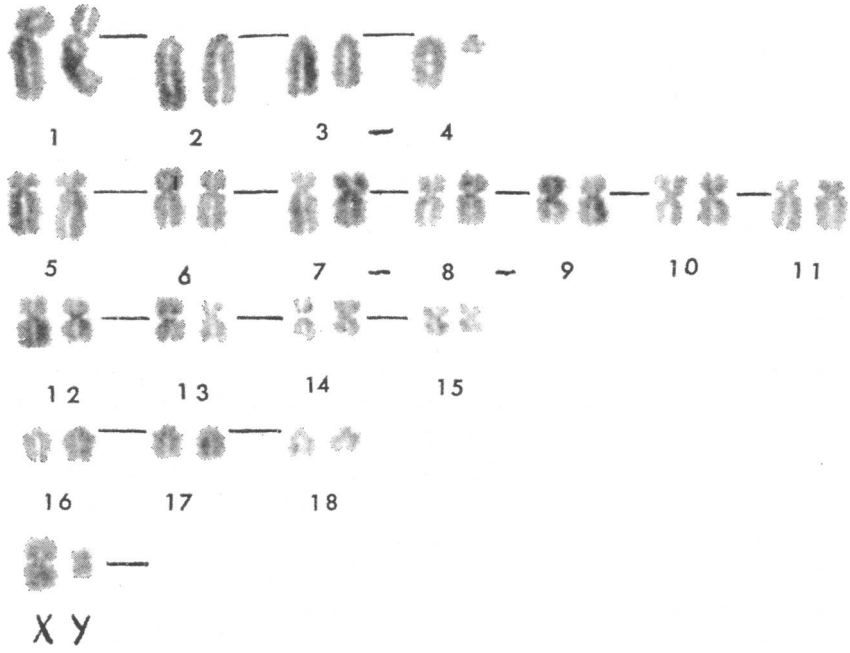
| Representation of abnormal karyotype | Deviation from expected karyotype | Number of individuals ♂ | ♀ | Fig. |
|---|---|---|---|---|
| 4, 14 + (14 + T) | —F, + 14 | 6 | 2 | 3 |
| 4 + (4 + F), 14 | —T, + 4 | 1 | 0 | 4 |
| 4 + 4, (14 + T) | —F, + 4 | 0 | 1 | 5 |
| (4 + F), 14 + 14 | —T, + 14 | 1 | 0 | 6 |
| 4, 14 + T, + (7 — 9) | —F, + (7 — 9) | 1 | 0 | 2 |

## DISCUSSION

No unbalanced karyotype was found among the 111 piglets served by the translocation heterozygote and investigated postnatally. If gametes with an unbalanced karyotype are formed in the spermiogenesis of this boar and if such gametes fertilize an egg, resulting embryos will probably die and disappear. Balanced gametes either of the normal type including 4 + 14 or of the translocation type F + T are evidently formed in great majority. Judging from the karyotypes of embryos and fetuses also unbalanced gametes are formed. Gametes including the combinations 14 + T, 4 + F, 4 + T, 14 + F and (7 — 9) + T have been




**F i g u r e 5.** Unbalanced karyotypes in embryos. 38 XX, (4+4, 14+T).




**F i g u r e 6.** Unbalanced karyotypes in embryos. 38 XY (4+F, 14+14).

formed. The four types first mentioned are all derived from the two chromosome pairs 4 and 14 involved in the translocation. The remaining type has only the T-element from these pairs, but it has in addition one chromosome from the chromosome groups 7 — 9. Whether this phenomenon is a consequence of the translocation cannot be decided. There are reports (*Grell & Valencia* 1964) of nondisjunction in other chromosome pairs than the translocated one.

The gamete type including 14 + T was detected in not less than eight out of 12 unbalanced karyotypes. It was found both in 22 days and 80 days old fetuses. This could be a sign of preferential formation of this combination during the meiosis. The explanation could, however, alternatively be that fetuses getting this combination are prone to later mortality. None of the remaining karyotypes was detected in fetuses being older than 28 days.

Unbalanced karyotypes were seen in 11 % of investigated embryos. The translocation heterozygote had a reduced litter size amounting to 34 % when compared to one normal boar. Only about one third of the fertility reduction will consequently be explained by the existence of unbalanced karyotypes in those stages of fetal development investigated. The original intention was to have more embryos in early development included in the material. Another four gilts were slaughtered 10 days after service, but the chromosome preparations from the embryos were unsuccessful. This stage of embryonic development is particularly interesting in view of the report of *Mc Feely* (1967), who found unbalanced karyotypes (mainly triploid) in 10 days old embryos with normal parents. The two embryo litters of this age which were successful in the present investigation showed no abnormal karyotypes.

Comparing the relationship between translocation heterozygotes and normal karyotypes in embryos and in postnatal individuals there will be a deficit of translocations in the latter category. The difference, about 7 %, is not significant in this limited material. The biological background for a possible real difference might be a changed position effect causing increasing embryonic mortality.

A considerable difference in fertility reduction between the boar described by *Henricson & Bäckström* (1964) and his son delt with in the present investigation should be noticed. The

fertility reduction amounted to 56 % in the father and 34 % in the son, both carrying the same translocation. Even if the normal boars used as comparison and the female component were different when judging the fertility of the translocation boars, the difference between the two is very probably real.

## REFERENCES

*Basrur, P. K., V. R. Basrur & J. P. W. Gilman:* A simple method for short term cultures from small biopsies. Exp. Cell Res. 1963, *30*, 229—235.

*Grell, R. F. & J. I. Valencia:* Distributive pairing and aneuploidy in man. Science 1964, *145*, 66—67.

*Henricson, B. & L. Bäckström:* Translocation heterozygosity in a boar. Hereditas (Lund) 1964, *52*, 166—170.

*Mc Feely, R. A.:* A direct method for the display of chromosomes from early pig embryos. J. Reprod. Fertil. 1966, *11*, 161—163.

*Mc Feely, R. A.:* Chromosome abnormalities in early embryos of the pig. J. Reprod. Fertil. 1967, *13*, 579—581.

*Moorhead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips & D. A. Hungerford:* Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. 1960, *20*, 613—616.

## SAMMANFATTNING

*Fosterdöd hos svin till följd av obalanserad kromosomuppsättning.*

En lantrasgalt som var heterozygot för en translokation har använts i 43 parningar med 30 suggor. I jämförelse med en normalgalt, som delvis använts till samma suggor, hade den förstnämnda galten en med drygt 30 % reducerad fertilitet. Fem olika typer av obalanserade kromosomuppsättningar påvisades hos foster efter translokationsheterozygoten. Eftersom ingen obalanserad karyotyp påträffades hos 111 avkomlingar efter födelsen, torde samtliga defekta karyotyper ha dött på fosterstadiet. 41 % av de postnatalt undersökta individerna hade samma translokationsdefekt som fadern.

*(Received February 25, 1971).*

Reprints may be requested from: The department of animal genetics, nutrition and hygiene, Royal Veterinary College, S-104 05 Stockholm 50, Sweden.