

# CHROMOSOMAL ABNORMALITIES AND EMBRYONIC DEATH IN PIGS

## Chromosomale Anomalien und Embryonaltdod in Schweine

### Anomalies chromosomiques et mortalité embryonnaire chez les porcs

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In the last decennia, artificial insemination (A. I.) in swine has made little or no progress and natural breeding still plays an important role in porcine reproduction. When reproductive failures have to be scrutinized under conditions of natural breeding, it is often very hard, if not impossible, to split up the male factor in its main components. Theoretically reduced fertility can be due either to impaired fertilization or to increased embryonic mortality (E. M.). Fertilization itself can be deficient either by inadequate intrauterine deposition of the semen during coition or by absence of ovum penetration. So we developed a simple experimental procedure to differentiate between coital impotence, lack of fertilization or increased E. M. in 13 belgian landrace boars with normal ejaculated spermatozoa at routine semen evaluation.

As a first orientation 4 gilts in spontaneous standing heat were used for each problem boar. Two of them were naturally bred by the boar, 2 were inseminated deep into the cervix with an undiluted fresh ejaculate from the same boar. All sows were sacrificed 2 days following insemination or service, ovulation points were counted, the ova flushed from the oviduct and examined for fertilization.

In 3 from the 13 boars so far tested only unfertilized ova were recovered both following natural breeding and A. I. In these boars the reproductive failure obviously was caused by an impaired fertilizing capacity of the spermatozoa although they looked normal at routine semen examination.

In 3 boars most of the ova were fertilized following A. I. but not following natural breeding. In these boars faulty ejaculation is probably the main factor.

In the remaining 7 boars, 90-100 % of the recovered ova were fertilized both after A. I. and mating. So an increased or complete embryonic loss was surmised.

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To test this hypothesis, 6 more sows were mated by each of those boars. Two sows were slaughtered 6 d. after service, 2 after 10 d. and 2 after 25 d. In the 6 d. group, the uterus was flushed with 100 ml TC. 199 medium, the blastocysts counted, individually fixed in acetic acid/methanol and stained with orcein. For 10 d. old embryos the method proposed by McFEELEY (1966) was used. In the 25 d. group the uterus was opened and the individual embryos cultured following trypsinization (0.25 % for 15 min. at 37°C). The cell suspension was grown in plastic flasks for 2-3 d. and proceeded for karyotyping using standard techniques; no banding technique was used.

From these 7 boars 3 well separated abnormalities associated with increased E.M. could be demonstrated in 4 males:

1. *Sticky anaphases* (1 boar): 40 % E.M. was already present in the 6 d. group but increased to 84 % at 10 d. and 100 % at 25 d. In the surviving embryos no metaphase plates could be found but practically all dividing cells were in anaphase. The chromosomes of the 2 daughter cells however were stucked together and the cells remained linked with each other by one or more chromosomal filaments.

2. *Arrested embryogenesis* (2 boars). Egg recovery was normal at 6 d. (86 % of C.L.). All blastocysts however were in the 4-8 cell stage and in most of them some degree of shrinkage and (or) fragmentation was present. At 10 d. and 25 d. no single blastocyst or embryo could be retraced. The fertilized ova thus did not develop beyond the 8-cell stage. Whether there is any chromosomal basis for this arrested mitotic activity could not be elucidated in this experimental set-up.

3. *Autosomal translocation* (1 boar). At 6 d. 90 % of the ovulated eggs were recovered from the uterus and had a normal cytologic aspect. At 10 and 25 d. 40 % resp. 60 % E.M. was demonstrated. From the surviving embryos 25 % had an autosomal translocation. Since no banding technique was applied, the individual chromosomes could not be identified with sufficient certainty. But, judging from the morphological aspect 1 of the second largest submetacentric pair and the third telocentric were involved.

In 3 boars foetal development was normal in any period examined. We believe that in these males, the reproductive problems reported by the owner were transitory in nature and probably caused by a temporary and reversible testicular degeneration which was spontaneously restored at the time of examination.

## ZUSAMMENFASSUNG

Es wurden 13 sterile Eber der belgischen Landrasse, deren Samenqualität normal befunden war, untersucht. Zuerst wurden pro Eber 2 Sauen gedeckt, 2 mit frischem Samen inseminiert, 2 Tage später geschlachtet und die Eizellen auf Befruchtung geprüft. Von 3 Ebern wurden bei den 4 Sauen nur unbefruchtete Eizellen zurückgefunden und kam also die Befruchtungsfähigkeit der Samenzellen in Frage. Bei 3 Ebern waren nur nach K.B. die Eizellen befruchtet was auf eine Kopulationsstörung hindeutete. Bei den 7 übrigen Ebern ergaben die beiden Befruchtungstechniken gute Resultate und wurde einen erhöhten Embryonaltod vermutet. Durch diese Tiere wurden, je Eber, 6 weitere Sauen gedeckt und die

Blastozysten oder Embryonen nach 6, 10 und 25 Tage untersucht. Bei 4 Ebern wurde einen stark erhöhten Embryonaltod festgestellt entweder infolge «sticky» Chromosomen (1), Stagnation auf 4-8 Blastomeren (2) oder autosomaler Translokation (1). Bei 3 Ebern war die embryonale Entwicklung normal und wurde eine spontane Heilung einer reversibelen Spermadegeneration vermutet.

#### RESUME

Une méthode pour la différenciation de troubles de fertilité chez 13 verrats sans anomalies visibles dans le sperme est décrite. Par verrat 2 truies ont été saillies, 2 inseminées, abattues 2 jours après et les ovocytes contrôlées pour fécondation. Chez 3 verrats les ovocytes furent infécondées suivant les 2 méthodes, donc la capacité de fertilisation du sperme fut déficiente. Chez 3 verrats la copulation fut déficiente comme seulement l'I.A. donnait des oeufs fécondés. Chez les 7 autres la fécondation fut satisfaisante aussi bien après saillie naturelle qu'après I. A. Dans ces cas 6 autres truies ont été fertilisées par verrat et abattues après 6, 10 et 25 j. Chez 4 verrats on trouva une haute mortalité embryonnaire à cause des «sticky» chromosomes (1), arrêt au stade de 4-8 blastomères (2) ou une translocation autosomale (1). Chez 3 verrats le développement embryonnaire étant normal, une régénération spontanée d'une dégénérescence testiculaire réversible ne peut pas être exclue.

#### REFERENCE

McFEELEY, R. A. (1966): A direct method for the display of chromosomes from early pig embryos. *J. Reprod. Fert.*, 11, 161-163.

