# Cytogenetic Screening of Pigs

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The cytogenetic screening of pigs, carried out using continually refined cytomolecular techniques, enables a precise diagnosis of chromosomal abnormalities, which cause developmental anomalies and considerably reduce the fertility (by several dozen to 100%) and performance parameters of breeding herds, resulting in substantial financial losses. Due to the potential spontaneous occurrence of chromosomal aberrations and the rapid spread of these genetic defects in the population, especially under artificial insemination conditions, it is necessary to perform cytogenetic monitoring of animals qualified for reproduction, which is an important criterion when formulating specific selection guidelines.

Keywords: karyotype abnormalities ; cytogenetic screening studies ; pigs ; fertility

## 1. Definition

Cytogenetic monitoring allows the identification and early removal of pigs affected by inherited karyotype defects from breeding herds. These abnormalities cause developmental anomalies, considerably reducing the fertility (by several dozen to 100%) and performance parameters of breeding herds, resulting in substantial financial losses. This mainly concerns reciprocal translocations, typical of pigs, which are highly prevalent (about 0.46%), generally occur de novo, and normally result in low breeding soundness of the carriers.

## 2. Introduction

One of the major issues facing pig breeders is karyotype defects, which considerably reduce fertility parameters (by several dozen to 100%) and thus the productivity of breeding herds, resulting in substantial financial losses. These anomalies are generally heritable, occur spontaneously in animals with normal conformation (and semen parameters), and their hidden nature allows them to spread rapidly in populations, especially through artificial insemination [1][2][3]. These factors justify the necessity of routine karyotype screening of pigs qualified for reproduction [4].

Reliable assessment of the porcine chromosome set and detailed identification of abnormalities are based on more and more precise research techniques. This allows the early elimination of animals carrying aberrations from breeding herds. These aberrations are most often reciprocal translocations, typical of pigs, which generally occur de novo, are highly frequent (about 0.46% in populations of cytogenetically monitored boars qualified for reproduction), and normally lead to low breeding soundness of the carriers [1][5][6][7][8][9][10][11]. It should be noted that in practice, cytogenetic analysis showing a normal karyotype provides breeders with an additional criterion to qualify sows and boars for reproduction [2][4][12][13].

The application of this indicator in selection is favored by the fact that the global costs of cytogenetic monitoring of breeding stock are distinctly lower than the financial losses connected with the use of sires with chromosomal defects for breeding. As no comparative analyses in this regard have been performed in recent years, these conclusions are formulated based on French estimates, which account for the costs of karyotype screening and the economic consequences of using boars carrying reciprocal translocations in AI (artificial insemination) stations [4]. Considering the incidence of reciprocal translocations, which was determined for the group of sires qualified for reproduction as 1/200, and taking into account that one cytogenetic analysis costs about 60 euros, the cost of identifying a karyotype defect was calculated to be about 12,000 euros (200 × 60, where 60 euros is the cost of a single karyotype analysis) [4]. In contrast, the global cost of using a translocation-carrying boar in an AI station is about 20,000 euros (calculated using the actual reproductive period of the translocation-carrying boar until it is diagnosed with reduced fertility, which is determined from its mating results; not earlier than after 4 months). During this period, such an animal will produce about 160 litters (40 litters per month), which means that the number of piglets not obtained over the 4-month reproductive period will total 640 (160 × 4, where 4 is the average reduction in the number of piglets per litter as a result of carrying the translocation) [4]. This causes breeders a loss of 19,200 euros (640 × 30, where 30 euros is the price of one piglet). Financial losses are much higher when the reciprocal translocation is spread by a purebred boar from the selection or multiplication levels of the production pyramid because 50% of his offspring will, again, carry this heritable chromosomal mutation [4].

The economic calculation presented above is a concrete argument for pig breeding organizations and breeders associations to systematically eliminate carriers of chromosomal anomalies from the population. This calculation also confirms the need for routine cytogenetic screening of breeding stock as part of the genetic improvement programs of breeds and lines to improve their fertility, which largely determines the economic efficiency of breeding herds. The highest effectiveness of these activities will be ensured by the early prevention of cytogenetic defects based on the general principle of qualifying those young animals for breeding herds, which are screened for karyotype normality before their reproductive use [4][12]. In some countries, identification of chromosomal aberration carriers and their elimination from breeding is an established standard or a legal obligation arising from the implementation of tasks related to the organization of farm animal breeding and reproduction and from sire evaluation and selection programs to ensure planned breeding progress [13][14][15][16].

## 3. Cytogenetic Screening of the Pig Population

Balanced chromosomal mutations, in particular reciprocal translocations associated with a drastic reduction of pig fertility, are seen as a major breeding and economic problem. Owing to this, many years ago, some countries introduced concrete selection guidelines to screen the karyotype of animals characterized by low breeding soundness or developmental abnormalities [4][12]. One example is the Scandinavian countries of Sweden and Finland, where numerous cases of reciprocal translocations were identified in the early 1990s as part of karyotype screening of animals selected based on breeding records that indicated a low number of piglets per litter (5 to 7 piglets) [17][18]. This allowed for the determination of the hypothetical frequency of the mutation carriers in the group of boars with reduced fertility (50%) and the estimation of the financial losses of USD6000 and USD25,000 incurred in one herd by sires carrying two different translocations [17]. Moreover, single cases of reciprocal translocations were identified during that period in pig populations raised in countries where no regular karyotype screening was conducted as part of national breeding evaluation programs, the result of which animals for cytogenetic tests were selected randomly or based on breeding recommendations associated with suspected developmental anomalies. One example is the reciprocal translocation that causes a slight decrease in fertility (5%), which was detected as part of pig herd monitoring in Germany (in the former GDR). In this case, the direct losses related to annual boar reproduction were estimated at around DDM28,000, and the global costs resulting from putting the offspring into breeding were much higher due to the accumulation of a small individual effect in a large population [18][19].

During the same period, at least several dozen new translocations were reported in France, where pig karyotype screening was included in the national system for the selection of breeds, lines, and individuals for high fertility [19][20]. As part of this program, in which the criterion was litter size, 800,000 litters sired by 20,000 boars were annually evaluated, and sires that produced fewer than 8 piglets in 6 successive litters were sent for cytogenetic analysis. In France, 42% of the boars with reduced fertility were then found to carry reciprocal translocations. In turn, assuming that the frequency of the boars with reduced fertility in the sire group was 0.15%, the frequency of the translocation carriers in the boar population was calculated to be 0.06% (one case per 1500 animals) [12][19][21]. Furthermore, in the 1990s, the PROSIM simulation model was used in France to analyze the economic consequences of reciprocal translocations using the example of a mutation that reduces fertility in the affected individuals by 45%. As part of this evaluation, financial outlays and revenue were compared in two equal herds of sows, one being mated to a translocation-carrying boar and the other to a boar with a normal karyotype. The direct farm losses from the failure to produce piglets (based on 65% mating success) were estimated at USD6000, and the losses resulting from the use of a translocation-carrying sire at the AI station was estimated to be USD105,000 (based on 650 semen doses per boar) [12][19][22]. In the United States, it was concluded based on estimates that the proportion of boars with low fertility was 3.7% (around 25 times that in France), which suggests the likelihood of a large number of translocation carriers. Therefore, in the 1990s, the US breeding program used the central boar selection system PIG CHAMP, based on similar assumptions as in France [18][19].

In other countries such as Hungary and The Netherlands, efforts were mainly concentrated on the cytogenetic screening of AI boars. As a result, several translocations were detected, and the frequency of chromosomal rearrangements among sires from Dutch AI centers was estimated to be 1.5%, which at that time was in excess of the expected value reported in the literature [4]. Additionally, in France, preliminary karyotype screening of 450 station boars showed the actual frequency of structural aberration carriers to be around 0.40%, much more than the hypothetical value reported earlier (0.06%), which was a strong argument for the intensification of screening tests when qualifying the boars for reproduction [4][23].

Additionally, in Poland, pig karyotype screening was not subject to any breeding rules, and such analyses (since the 1990s) could be performed only as part of boar reproductive performance tests. During that time, several hundred pigs that were randomly chosen from the population (using no fertility data, which are an indication for karyotype assessment) were subjected to screening, including only several dozen breeding and AI boars. This screening revealed the first reciprocal translocations that reduced fertility, the most fateful of which was translocation t(7;13)(q13;q46), which reduced

the mean number of piglets per litter by 48% [12][19][24]. The simulated calculation of the economic consequences of carrying this defect showed that the financial losses caused by using one carrier boar in a herd are around USD8000 for natural mating, and USD162,000 for artificial insemination in the active population. These estimates are based on the loss of profit on a pig farm with an annual production of 3380 heads, or the loss of gross trade value from the sale of 1560 pigs, or the loss due to mortality of 11,267 day-old piglets [12][19].

The monitoring results from the 1990s encouraged other breeding centers to launch cytogenetic testing of boars from the reproductive sector. In Spain, based on the initial results of these analyses, the frequency of chromosomal abnormalities and structural anomalies in a commercial herd of more than 700 sires was estimated to be 3.8% and 3.3%, respectively [25][26][27][28][29][30]. In Canada, the frequency of karyotype defects among almost 900 boars qualified for reproduction in 2016 was estimated at 1.64% (with 1.36% animals carrying a translocation). Extrapolating this to a commercial scale, it was estimated that aberration-carrying piglets would occur in over 46,400 litters (out of around 2.9 million litters produced per year), which means an average loss of 4 piglets per litter. With the price of 25 CAD per piglet, this gives an annual loss of CAD4.6 million [31][32][33]. The results of this prediction analysis formed the basis for including almost 6000 boars (from Canadian breeding centers) in the program for cytogenetic screening and eradication of aberration carriers, which, in 2018, reduced the incidence of chromosomal mutations down to 0.91% [34][35].

The gradual expansion of artificial insemination in pig reproduction has increased the interest in cytogenetic monitoring of young boars qualified for reproduction in breeding centers. In some countries, considering the consequences of the high incidence of reciprocal translocations and the magnitude of financial losses, it was decided that systemic solutions for karyotype screening of all young boars before their use in AI stations would be adopted [4]. These activities are exemplified by the launching, more than 20 years ago in France, of a commercial cytogenetic platform at the l'Ecole Nationale Vétérinaire in Toulouse (ENVT–INRA), certified with the ISO 9001 standard since 2012, which allows large-scale monitoring of French pig populations [13][36][37]. The basic principle of the platform is to monitor the karyotype of young purebred boars and terminal crosses (aged 6 to 10 months) prior to use in AI stations at the request of breeders associations [4][13][15][21][23][38]. It should be noted that this laboratory concurrently performs cytogenetic screening of sires with reduced fertility parameters, and before that, it performed similar tests for the needs of breeding centers from other European countries (The Netherlands, Belgium, Germany, Spain) [13][38]. The effectiveness of the platform is evidenced by the data published in 2016–2018, according to which 39,000 boars were screened (up to 2000 per year) and over 180 cases of structural chromosomal abnormalities were identified [13][39][36]. Among the diagnosed aberrations, reciprocal translocations formed the overwhelming majority (87%), followed far behind by inversions (10%), Robertsonian translocations (2%), and other structural anomalies (1%). Apart from the structural rearrangements, chromosome aneuploidies, XX/XY cell chimerism, and sex-reversal cases were also diagnosed. For many years, the frequency of balanced chromosomal mutations has remained almost unchanged at around 0.5% (including reciprocal translocations 0.46%), and the studies did not reveal any breed trends in relation to the incidence of these changes, which confirmed their random nature [13][39][36]. As this type of rearrangement can generally lead to serious reproductive disorders (considerable reduction in litter size or infertility), it can be assumed that the ENVT–INRA program (operated in France since 1997) has saved pig producers from huge economic losses over the last 20 years. Additionally, in Poland, over the last 10 years, cytogenetic screening has included a group of several hundred young boars qualified for breeding, and the incidence of structural chromosomal rearrangements in this population, similar to France, was determined to range from 0.46% to 0.47% [12].

Recently, several European countries (The Netherlands, Spain, Sweden) that have intensified pig production through artificial insemination (using semen doses from a single sire) have emphasized the importance of boar karyotype screening as one of three components of an additional package of tests qualifying young boars for reproduction in AI centers. According to this concept, the estimated cost of implementing this package (in vitro fertilization test, assessment of nuclear chromatin, cytogenetic analysis) in an AI station with 100 boars would be around 100 euros per sire; this would allow an increase in mean litter size by 0.1 piglets and improve the economic effect by around 0.7 million euros [40].

It should be highlighted that the introduction of modern cytomolecular techniques into laboratory practice has considerably increased the diagnostic potential of the screening system, resulting in a marked increase in the number of karyotype abnormalities identified [40][41]. To date, cytogenetic screening of many pig populations around the world has resulted in the identification of over 220 structural karyotype defects, including almost 200 reciprocal translocations with a clear negative impact on fertility and economic efficiency of production [1][2][13][34][42][43][39][33][44][29][30][35][40][45][46][47][48]. It seems that the elaboration and implementation of the next screening strategies will significantly intensify the detectability of reciprocal translocations, which are likely much more frequent in breeding populations than previously estimated.

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