

Case Report

A case of impaired fertility in a Large White boar with sex chromosome chimerism

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Abstract

Chromosomal abnormalities are a well-established cause of fertility impairment in domestic animals. Chromosomal abnormalities in pigs may result in low prolificacy and subsequent reduction in reproductive parameters (e.g. litter sizes and nonreturn rates) having substantial impact on swine industry. Until now, the effect of sex chromosome abnormalities on fertility has not been thoroughly documented for male pigs. We report a case study involving a Large White boar with sex chromosome chimerism (2n = 38, XX/XY) identified by conventional karyotyping and fluorescence in-situ hybridization. Progeny of this boar was evaluated; there was reduction ($p < 0.001$) in litter size demonstrating the potential impact of sex chromosome chimerism on herd productivity in pigs.

Keywords: Male pigs, chromosomal abnormalities, subfertility

Introduction

Chromosomal abnormalities are a common cause of infertility in mammals, including several species of domestic animals. In pigs, alterations in chromosome structure (mainly, reciprocal translocations and to a lesser extent inversions) and sex chromosome numerical abnormalities (usually mosaic)^{1,2} resulted in poor fertility due to embryo or fetal loss causing reduction in prolificacy and subsequent impact to farming industry.³ Chromosome abnormalities that lead to low prolificacy can be compatible with a normal phenotype, albeit some can lead to demonstrable infertility (e.g. compromised semen parameters or complete sterility),⁴ whereas others are only identified when litter size or nonreturn rate data are analysed or through a proactive screening program.^{2,5}

Blood cell sex chromosome chimerism (XX/XY) originating due to vascular placental anastomosis in utero has been demonstrated as a cause of complete infertility in females of several species, including cattle,⁶ sheep,⁷ goats,⁸ and pigs.⁹ Although controversial, XX/XY blood cell chimerism has been deemed not to produce substantial effect on fertility in males.^{10,11} However, a study in bulls suggested that chimeric males may have a reduction in fertility, potentially due to poor sperm quality.¹² Sex chromosome abnormalities have

been related to subfertile phenotype in male pigs,¹ although in several cases they might present a normal phenotype.¹⁰ Interestingly, fertility data from affected boars are scarce and seldom reported.

Recently, an increased incidence of XX/XY blood chimerism in pigs has been observed and linked to the selection of highly prolific lines,⁹ making investigation into the effects of sex chromosome chimerism on male fertility crucial. We present a case of a Large White XX/XY chimeric boar from a commercial herd whose reproductive parameters were tracked to assess the impact of this condition on progeny.

Materials and methods

A total of 66 boars, from a large Canadian commercial swine operation (Hypor Incorporated, Regina, Canada) were karyotyped by Karyotekk Incorporated (Guelph, Canada) as part of the prebreeding screening program (2021-2022). Whole blood was collected by trained veterinarians and processed for karyotyping by standard procedures.³ This led to the identification of an XX/XY sex chromosome chimeric boar. Additionally, fibroblast cultures from a ear skin biopsy sample from the chimeric boar, provided by the owner, were

processed for cytological analysis by Karyotekk Incorporated by standard procedures.¹³

Fluorescence in situ hybridization (FISH) was performed using a X chromosome probe labelled with fluorescein isothiocyanate provided by CytoScreen Solutions (University of Kent, Canterbury, UK) and applied to lymphocytes and skin fibroblasts¹⁴ to determine the degree of chimerism in hematopoietic and nonhematopoietic cells.

Reproductive parameter data from 66 boars, including 1 chimeric boar collected on the farm from January 2021 to January 2022 were analyzed. Parameters of interest included litter size, total live born, and stillborn. Boars ($n = 65$) with no known chromosome abnormality were assessed for mean litter size (total number of piglets divided by total number of litters), and mean live born and stillborn rates. Mean litter sizes were compared using the R programming language (R Core Team, Vienna, Austria, v3.6.0). Z-test was employed to compare the chimeric boar reproductive parameters with the farm population.

In accordance with the Canadian Council for Animal Care and the University of Guelph Animal Care committee a review of the research protocol was not required.

Results

Cytogenetic analysis

Examination of the GTG-banded karyotypes obtained from blood leukocytes of the proband revealed a male ($2n = 38$,

XY) and a female ($2n = 38$, XX) karyotype (Figure 1) and thus confirmed blood cell chimerism ($2n = 38$, XX/XY).

In hematopoietic tissue (leukocytes), FISH technique revealed that 55% of cells with one fluorescence signal, suggesting a $2n = 38$, XY karyotype (Figure 2-A), and 45% of leukocytes with 2 fluorescence signals suggesting $2n = 38$, XX karyotype. In nonhematopoietic (fibroblasts) tissue (Figure 2-B), a low proportion ($< 7\%$) of $2n = 38$, XX were detected (Table 1).

Reproductive parameters

Litter sizes were different between the chimeric and control groups (11.91 versus 15.04, $p < 0.0001$) as were live born rates (10.32 versus 13.55, $p < 0.0001$), whereas stillborn rates were not different (Table 2).

Discussion

Effect of blood chimerism (XX/XY) on male fertility has not been thoroughly documented in pigs. There were no reproductive impairments in 7 XX/XY 'phenotypically normal boars';¹⁰ however, anecdotally, reduced litter size has been observed.¹⁴ Although this study was focussed on a single boar and is thus not a systematic overview, it provided an evidence that XX/XY chimerism can be related to subfertility.

The exact mechanism responsible for reduced fertility in this case has not been identified; however, it could be similar to previous reports that described poor semen

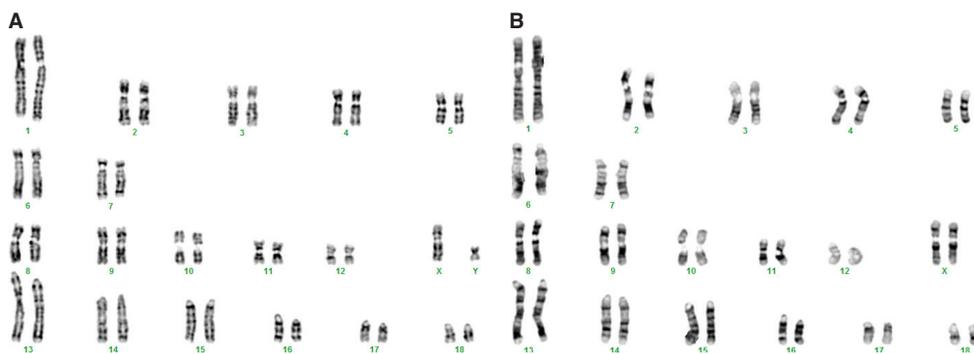


Figure 1. Karyotypes of lymphocyte metaphases from the chimeric boar (A. $2n = 38$, XY and B. $2n = 38$, XX)

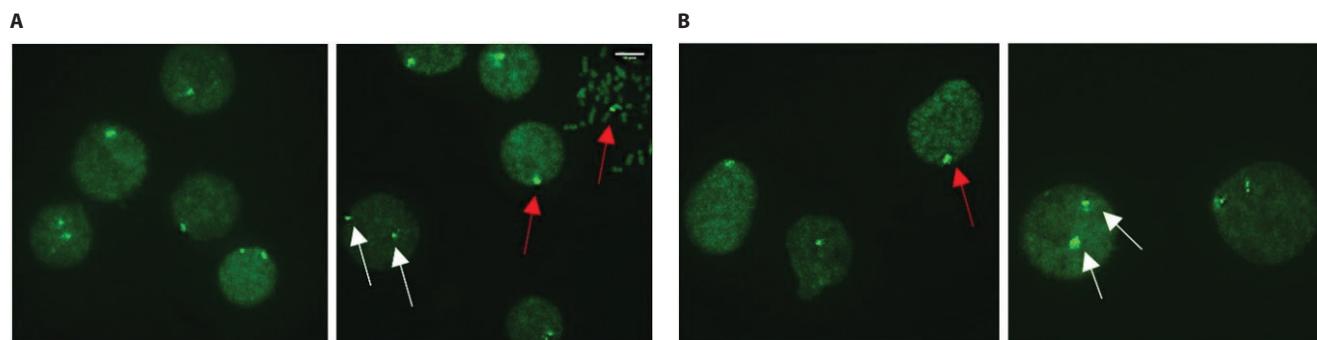


Figure 2. FISH-stained cells from the chimeric boar from A. lymphocytes and B. fibroblasts. White arrows indicate female (XX) nuclei and red arrows indicate the single X chromosome in an XY nucleus

Table 1. Distribution of XY and XX cells on hematopoietic and nonhematopoietic tissues

Type of cell	2n = 38, XY	2n = 38, XX	Percent XX
Hematopoietic (lymphocyte culture)	115	85	42.5
Nonhematopoietic (fibroblast culture)	186	14	7

Table 2. Reproductive parameters of chimeric boar and the population of boars of the farm

Reproductive parameter	All boars (n = 65) 1,243 litters	Chimeric boar 34 litters	p value
Litter size (mean ± SEM)	15.04 ± 0.103	11.91 ± 0.594	< 0.0001
Live born (mean ± SEM)	13.55 ± 0.098	10.32 ± 0.607	< 0.0001
Stillborn (mean ± SEM)	1.49 ± 0.051	1.58 ± 0.443	0.3775

quality in chimeric bulls.¹² In pigs, chimerism often extended to nonhematopoietic tissues, including, lung, hair, and skin, although at a low rate (< 8%).¹⁰ In agreement with that study, we identified a low percentage (7%) of XX cells in skin fibroblasts. Chimerism in somatic tissues might relate with the reduction in fertility observed in this boar.

Sex chromosome chimerism is currently considered the most common intersex condition in pigs. It has been suggested to be related to large litter sizes where uterine crowding may cause an increase in placental anastomosis between litter mates.⁹ Large White boar is a prolific breed with an average litter size of 13.4 piglets.¹⁵ Average litter size of the herd in this investigation was 15.04 total born and 13.55 born alive that included chimeric boar's dam, whose average over 6 litters was 15.3 total born and 14.8 born alive suggesting that this factor might have a role in this case.

To our knowledge, ours is among only a few cases that reported the fertility of a chimeric boar. This report demonstrated that chimerism might also have detrimental effects on male fertility in pigs. Considering the available information, it may be advisable to pay particular attention to XX/XY chimeric boars in breeding programs to obtain additional data regarding the effects of this condition on fertility. Proactive routine cytogenetic screening involving conventional karyotyping and FISH,^{2,5} can identify these and other problems before an animal enters the breeding program.

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Conflict of interest

None of the authors has any conflict of interest to disclose.

Author contributions

JP performed data analysis and wrote the manuscript. LC contributed to data analysis and karyotyping. LL contributed to sample processing, images, and evaluation. MM contributed to manuscript preparation, DV performed karyotyping analysis. DG edited the manuscript and provided the FISH probe. LF contributed to editing the manuscript and supervising students involved in the project. PC provided reproduction data. AK edited the manuscript, supervised students involved in the project, and provided funding. All authors have read and approved manuscript submission.

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