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Cytoscreening of Indigenous pigs for Numerical Chromosomal Abnormalities

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Abstract

The chromosomal abnormalities are found almost in all the domestic animal species. They reduce not only the production performance but also the reproduction potential of the animal. The present study was designed to investigate the indigenous pigs for the various chromosomal abnormalities. For the present investigation twenty indigenous pigs 10 males and 10 females of same generation and age from the AICRP unit of Pigs, at IVRI Bareilly were selected. Apparently healthy animals and also those with the low litter size (<6) and failure to conceive were used for this study. These animals were screened for numerical chromosomal abnormalities if any, by karyotyping.

Preparations of chromosomes for cytoscreening of animals was done on the basis of short-term whole blood lymphocyte culture technique as described by Moorhead and then the karyotypes of animals were prepared using cells arrested at metaphase division. Ten metaphases were used for karyotyping per animal. So, in this study a total of 200 metaphase plates were used for screening 20 animals. All the animals were found to be devoid of any numerical chromosomal abnormality (aneuploidy and euploidy) as per cytoscreening.

Keywords: Chromosomal abnormalities, aneuploidy, blood lymphocyte culture, metaphase plate

1. Introduction

Animal husbandry, an important sub-sector of Indian agriculture is a key contributor of rural economy. The livestock sector contributes 4.1% to the national economy (in terms of GDP) and 26.02% to the Agricultural GDP [1]. In India total livestock population is 512.05 million with 10.29 million pigs, constitute 1% of world pig population and 2.01% of total Indian livestock population [2]. Pig rearing is one of the important occupations of rural poor and weaker sections of the society, mainly the tribal masses of India. It is a low or zero input rearing system and has immense potential to ensure nutritional and economic security for the weaker sections of society. It is the most efficient way of meat production due to important inherent traits which includes high fecundity; early maturity, short generation interval, high feed conversion efficiency and growth rate, relatively small space requirement, and this can be maintained with low quality feed.

The world population is rising rapidly, which may exceed 9 billion by 2050 and to feed these growing masses there is huge pressure on livestock to increase the production [3]. Reproduction being the backbone for production, the factors affecting reproduction must be taken care of, as the need for efficient large-scale breeding and productivity improvement is becoming more important day by day than ever before. Several reproductive traits such as ovulation rate, embryo survival, number born, number weaned and age at puberty in the female and testicular size and libido in the male are important for the productivity. The economics of pork production greatly depends on the factors such as fertility and litter size [4]. As embryonic loss during pregnancy has major impact on litter size, reduction of this loss is the key to improve this trait [5]. The major etiologic factors in the risk of embryo malformations and early embryonic mortality in the domestic pigs are chromosomal abnormalities contributing to about one-third of the early embryonic mortality in normal pigs [6, 7].

The deviations from the standard chromosome number and morphology of a species are referred to as chromosomal abnormalities or chromosomal aberrations. A variety of sex chromosome abnormalities have been described in domestic livestock species [8, 9, 10, 11]. These can be classified broadly into two classes (Mosaic and Non-mosaic). The majorities are in mosaic form, where chromosomally normal cells of a particular sex contribute to the phenotype together with cells containing sex chromosomes of other sex, thus blurring the

dosage effect. The other type is non-mosaic aneuploidies, which cause either haplo-insufficiency, such as X-monosomy, or that increase the dosage, such as XXX, XXY and XYY genotypes [12,13]. On the other hand these chromosomal abnormalities in form of deletion and duplication (structural changes) not only alter gene function but also the gene expression.

The importance of cytogenetic screening for prevention of infertility caused by chromosomal anomalies in a modern breeding system has been established since 1980s [14]. For any country with an intensive pork industry, the cytogenetic screening programs to systematically test all breeding boars serve an essential tool for swine improvement as the sires with the chromosomal abnormalities have low or reduced reproductive performance [15]. To minimize the risk of carriers of chromosome aberrations entering artificial insemination centres, systematic cytogenetic screening of potential breeding boars should be done.

2. Material and methods

2.1. Experimental Animals

For the present investigation about 20 indigenous pigs, 10 males and 10 females of same generation and age from the AICRP unit of Pigs, at IVRI Bareilly were selected. Apparently healthy animals and also those with the low litter size (<6) and failure to conceive were used for this study. These animals were screened for numerical chromosomal abnormalities if any, by karyotyping.

2.2. Collection and Storage of sample

Blood samples (2ml) for cytoscreening were collected from the selected pigs aseptically into vacutainers (Becton and Dickinson, USA) containing heparin as anti-coagulant from the anterior vena cava by holding the animal in dorsal recumbency. After collection of blood; the tube was tightly capped, shaken gently, kept immediately in an icebox containing gel cool pack and transported to laboratory without delay. After reaching the laboratory, the blood samples for cytoscreening were used for setting of culture within 2 hrs of collection.

2.3. Cytogenetic screening of crossbred pigs using karyotype

Short-term whole blood lymphocyte culture technique with slight modifications, suitable to our laboratory conditions was followed for the preparations of chromosomes [16]. The technique followed as per the following steps:

2.4. Preparation of Glassware

The glasswares used were made grease free and sterilized to avoid contamination and loss of cells. It was properly cleaned, dried, autoclaved, sprayed with spirit and then kept in Laminar flow hood. The various actions taken are briefly described in following steps.

2.4.1. Cleaning and Rinsing

All glass equipments were kept in chromic acid solution overnight, and washed thoroughly first with running tap water and then rinsed with filtered (Water Pro/RO) water and finally dried in hot air oven at 150°C for 2-4 hours.

2.4.2. Sterilization

All the glassware and media filter assembly used in preparation of media and setting up of cultures was sterilized

in an autoclave for 20 minutes at 15 lbs pressure. The glassware was kept in laminar airflow hood fitted with an ultra violet tube after spraying with spirit.

2.4.3. Preparation of culture medium

The complete medium (ready to use) was prepared under sterile conditions in the laminar airflow cabinet. In a clear conical glass flask (capacity 1000ml) 500 ml Milli - Q water/autoclaved water was taken. RPMI Medium 1640 8.1 gm (@ 1.62gm/100ml Milli - Q water) was added in flask and mixed by slight shaking. Antibiotics (streptomycin and penicillin), Lectin PHA, Pokeweed mitogen and concanavalin A (@ 1ml/100ml Milli - Q water) each was added in it. The flask was swirled gently to dissolve all the chemicals completely. The pH of the medium was adjusted to 7.2 by adding drop-by-drop sterilized NaHCO₃ solution (4.4%). The prepared medium was sterilized by filtering through millipore membrane (0.22 µm) fixed assembly. After filtration, 100 ml Fetal Bovine serum (FBS) was added to medium in laminar hood. One drop of sterile sodium heparin was added to prevent the coagulation problems. Gentle swirl was given for proper mixing. This complete culture medium (CCM) was distributed in screw capped culture bottles (30 ml) in aliquots of 6 ml each. The culture bottles were stored effectively up to 3 months under frozen condition (-20°C), used as per need, however, thawed to 37° C in incubator before use.

2.4.4. Setting-up of cultures

The ultraviolet lamp of the laminar airflow cabinet was switched on one hour prior to setting the cultures after mopping the working areas with 70 percent ethanol. All the chemicals required i.e., RPMI 1640 with L-glutamine, Phytohaemagglutinin (PHA-M), Fetal Bovine Serum (FBS) and Antibiotics; colchicines solution were taken out of deep freezer (-20 °C) and kept at room temperature for thawing. The ultraviolet lamp was switched off and the work area of laminar airflow cabinet was sprayed again with 70 percent ethanol. The thawed culture bottles were taken into the laminar airflow bench after spraying with 70 percent ethanol and mopping. The caps of the vacutainer tubes containing blood samples were wiped with 70 percent ethanol and mopped with paper towel. Duplicate cultures were set up for each animal.

Whole blood cultures were established either in freshly prepared medium or stored medium (-20 °C). If the culture medium was in frozen state, then the bottles were kept in an incubator (37.5 °C ±0.5 °C) and were brought the medium to room temperature. The blood samples collected in vacutainer tubes were given a mild shaking by inverting the tubes a few times and after spraying with spirit kept in laminar airflow hood. With the help of sterile syringes, 0.7 ml of blood was taken out from the vacutainer tubes and transferred to the culture bottles containing 5 ml culture medium. The contents were given a gentle swirl for quick mixing.

The cap and the mouth of the culture bottles were flamed over the spirit lamp before making it airtight to reduce the chance of bacterial contamination. Identification marks like date; animal number, etc. were written on the stand corresponding to each culture bottle and was also recorded in the laboratory notebook. The culture bottles were transferred to an incubator at 37.5 °C (±0.5 °C) for a period of 72 hours with mixing for 15 min, twice daily.

2.4.5. Harvesting of the Culture

After incubating for 72 hours, 2 drops (0.5µg/ml) of colcemid solution (Himedia) was added to each culture bottle, and again kept in the incubator. Cultured contents were transferred to centrifuge tubes (15 ml) after 45 minutes of incubation with colcemid. These tubes containing cultured cells in medium were centrifuged for 20 minutes at 2000rpm. The supernatant was discarded carefully leaving cell suspension in the bottom of the centrifuge tubes in the form of pellets. The pellets in each tube were treated with 7ml warm (37.5 °C ±0.5 °C) hypotonic solution (0.075 M KCl, 1.667 gm KCl in 300 DW), kept at 37°C (needed about 7 ml/sample) for 20 minutes. The pellets were mixed gently with Pasteur pipette and then centrifuge tubes were transferred again to the incubator at (37.5 °C ±0.5 °C) for another 20 minutes. Later on, 1 ml of chilled fixative was added to each centrifuge tube and mixed gently. Fixative solution: 3 Methanol: 1 acetic acid (keep in freezer) (needed about 15 ml/sample). Subsequently, centrifugation was done for 20 minutes at 2000 rpm, after which the supernatant was discarded and pellets were resuspended in 5 ml chilled fixative. This procedure was repeated thrice. After third centrifugation, supernatant was removed with the help of Pasteur pipette judging the button size. The pellet was mixed by gentle pipetting to obtain a slightly milky cell suspension.

2.4.6. Cleaning of Slides

The slides were dipped overnight in chromic acid solution, and rinsed thoroughly in running tap water and then with filtered pure water. The slides were stored in a plastic bottle containing absolute methanol and added con 5% HCl and kept in the refrigerator. Before use, slides were taken out, dried and cleaned thoroughly with a clean muslin cloth.

2.4.7. Preparation of chromosome slides

On a clean slide, about 3-4 drops of cells suspension were dropped from a height of about 2 feet. The slides were flamed fixed by bringing the slide into momentary contact with a flame. Then the slides were placed on filter paper and allowed to dry at room temperature for 10-15 minutes. Subsequently, the slides were placed in a slide box for storage until staining was carried out.

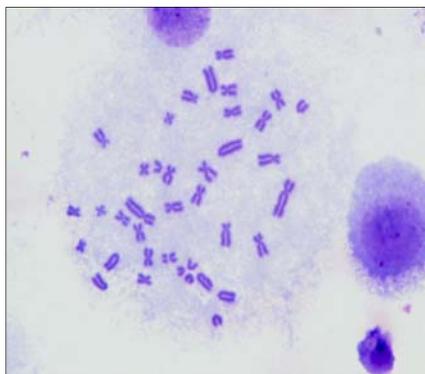


Fig 3.1: Metaphase plate of female

2.4.8. Staining of slides

The slides were stained with freshly prepared 2% Giemsa stain solution in coupling jar for 20 min followed by washing with distilled water and air-drying. After complete drying, slides were mounted with DPX and dried in incubator at 37 °C for 48 to 72hrs.

2.4.9. Screening of slides, microphotography and karyotype preparation

The slides were screened and photomicrographed under microscope with standard light optics with 10-x objective and a closer look at chromosomes with the 40-x objective and photography with 100-x oil immersion objective.

The prints of metaphase chromosomes were used for making karyotype. The chromosomes were counted and numbered on the back-side (body of chromosome) of the print keeping against bright light. The homologous pair of chromosomes identified, were cut by matching their absolute length in the print. These pairs were arranged on a sheet of paper, in a decreasing order of their length and also based on the morphology, as per International System for the Cytogenetic Nomenclature of Domestic Animals. After arranging the chromosomes as accurately as possible with the help of a dial calipers the chromosomes were pasted on a paper in pre printed format. Ten metaphases were used for karyotyping per animal.

3. Results and Discussions

The chromosomes at the metaphase stage are thickest with their sister chromatids being distinct. Metaphase is therefore, the ideal stage for the study of number and morphology of chromosomes.

3.1. Modal chromosome number

In the present study, the standard protocols followed for short-term lymphocyte culture method with minor modifications gave satisfactory results in terms of the quantity and quality of metaphases obtained. Chromosomes of ten well spread metaphases from each animal with a total of 200 metaphases from 20 indigenous pigs were examined and it was found that all metaphases contained a diploid chromosome number (2n) of 38 with XY complement in males and XX complement in females, indicating absence of any numerical chromosomal abnormality [17,18]. The Giemsa-stained chromosomes seen at an arrested metaphase plate are presented in Fig. 3.1 and 3.2.

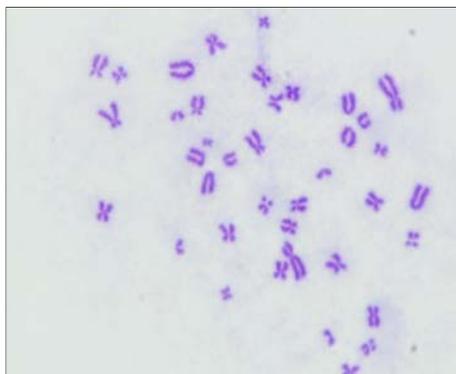


Fig: 3.2: Metaphase plate of male pig

3.2. Chromosome morphology

The karyotypes prepared from the Giemsa-stained metaphases of both indigenous male and female pigs are presented in Fig.

3.3 and 3.4 respectively. The pairing of chromosomes was based on their length and arranged in the descending order of their length. Out of 19 pairs of chromosomes found in the

indigenous pigs, there were 18 pairs of autosomes and one pair of allosomes. First 5 pairs of autosomes were submetacentric, next two pairs were sub telocentric (6-7), subsequent 5 pairs were metacentric (8-12) and remaining six pairs were telocentric (13-18). First chromosome was the longest pair and thirteenth pair was the second longest, while

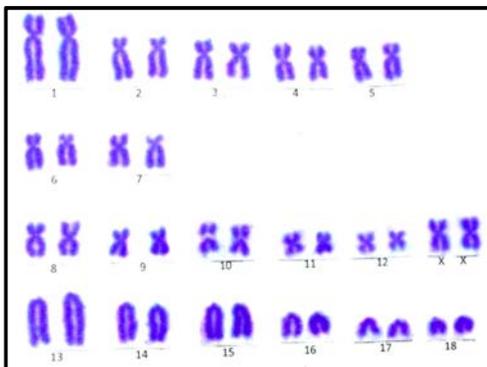


Fig 3.3: Karyotype of female pig

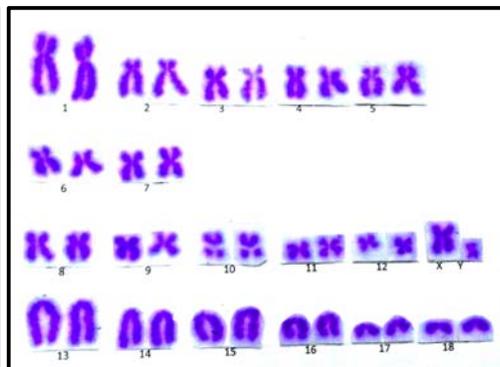


Fig 3.4: Karyotype of male pig

4. Conclusions

As the various chromosomal aberrations directly affect the reproduction parameters and thereby causing loss to the various animal industries, removing the carriers from reproduction will prevent not only the dissemination of the chromosomal rearrangements in the off-spring but will also save the cost of raising the carrier animals.

5. Acknowledgement

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6. References

1. Anonymous. BAHS, Ministry of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India. 2014, 1-15.
2. Anonymous. 19th Livestock Census. All India Report, DAHD and F, Ministry of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture Government of India. 2014, 34-35.
3. Foods and Agriculture Organization of the United Nations (FAO), 2016. www.fao.org/ag/againfo/themes/en/meat/background.htm 1 Meat consumption. Accessed 12 Oct,
4. Rothschild MF. Genetics and reproduction in the pig. *Animal Reproduction Science*. 1996; 42(1):143-151.
5. King WA. Chromosome abnormalities and pregnancy failure in domestic animals. *Advances in veterinary science and comparative medicine*. 1990; 34:229-250.
6. Akesson A, Henricson B. Embryonic death in pigs caused by unbalanced karyotype. *Acta veterinaria Scandinavica*. 1972; 13(2):151.
7. Mcfeely RA. Chromosome abnormalities in early embryos of the pig. *Journal of reproduction and fertility*. 1967; 13(3):579-581.
8. Villagómez DAF, Parma P, Radi O, Di Meo G, Pinton A, Iannuzzi L *et al*. Classical and molecular cytogenetics of disorders of sex development in domestic animals.

Y-chromosome was the smallest in the karyotype of the pig [19]. The X chromosome was metacentric, but not readily distinguishable from the autosome pair number 8, while Y chromosome was metacentric and found to be the smallest member of the karyotype. The identification of Y chromosome was depending on the earlier literatures [20].

- Cytogenetic and genome research. 2009; 126(1-2):110-131.
9. Pinton A, Ducos A, Berland H, Seguela A, Brun-Baronnat C, Darre A *et al*. Chromosomal abnormalities in hypoproliferative boars. *Hereditas*. 2000; 132(1):55-62.
 10. Gustavsson I, Settergren I. Reciprocal chromosome translocation with transfer of centromeric heterochromatin in the domestic pig karyotype. *Hereditas*. 1984; 100(1):1-5.
 11. Long SE. Reciprocal translocations in the pig (*Sus scrofa*): a review. *The Veterinary record*. 1991; 128(12):275-278.
 12. McFee AF, Knight M, Banner MW. An intersex pig with XX/XY leucocyte mosaicism. *Canadian Journal of Genetics and Cytology*. 1966; 8(3):502-505.
 13. Breeuwisma AJ. A case of XXY sex chromosome constitution in an intersex pig. *Journal of reproduction and fertility*. 1968; 16(1):119-121.
 14. Gustavsson I, Johansson I. Chromosome aberrations and their influence on the reproductive performance of domestic animals-a review. *Zeitschrift fur Tierzucht und Zuchtungsbiologie*. 1980; 97(1-4):176-195.
 15. Ducos A, Revay T, Kovacs A, Hidas A, Pinton A, Bonnet-Garnier A *et al*. Cytogenetic screening of livestock populations in Europe: an overview. *Cytogenetic and genome research*. 2008; 120(1-2):26-41.
 16. Moorhead PS, Nowell PC, Mellman WJ, Battips DT, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. *Experimental cell research*. 1960; 20(3):613-616.
 17. Gustavsson I. Standard karyotype of the domestic pig. *Hereditas*. 1988; 109(2):151-157.
 18. Hansen-Melander Eva, Melander Y. The karyotype of the pig. *Hereditas*. 1974; 77(1):149-158.
 19. Kanadkhedkar HL, Nehete SB, Suryawanshi AR, Umrikar UD. Chromosomal study of desi pig. *The Journal of Bombay Veterinary College*. 2006; 14(1-2):78-79.
 20. Vijn RK, Sahai R, Sharma A. Chromosomal profile of Indian domestic pig. *Indian Journal of Animal Sciences*. 1990; 60(11):1373-1376.