



## CYTOGENETIC INVESTIGATIONS IN SUBFERTILE BUFFALO FEMALES

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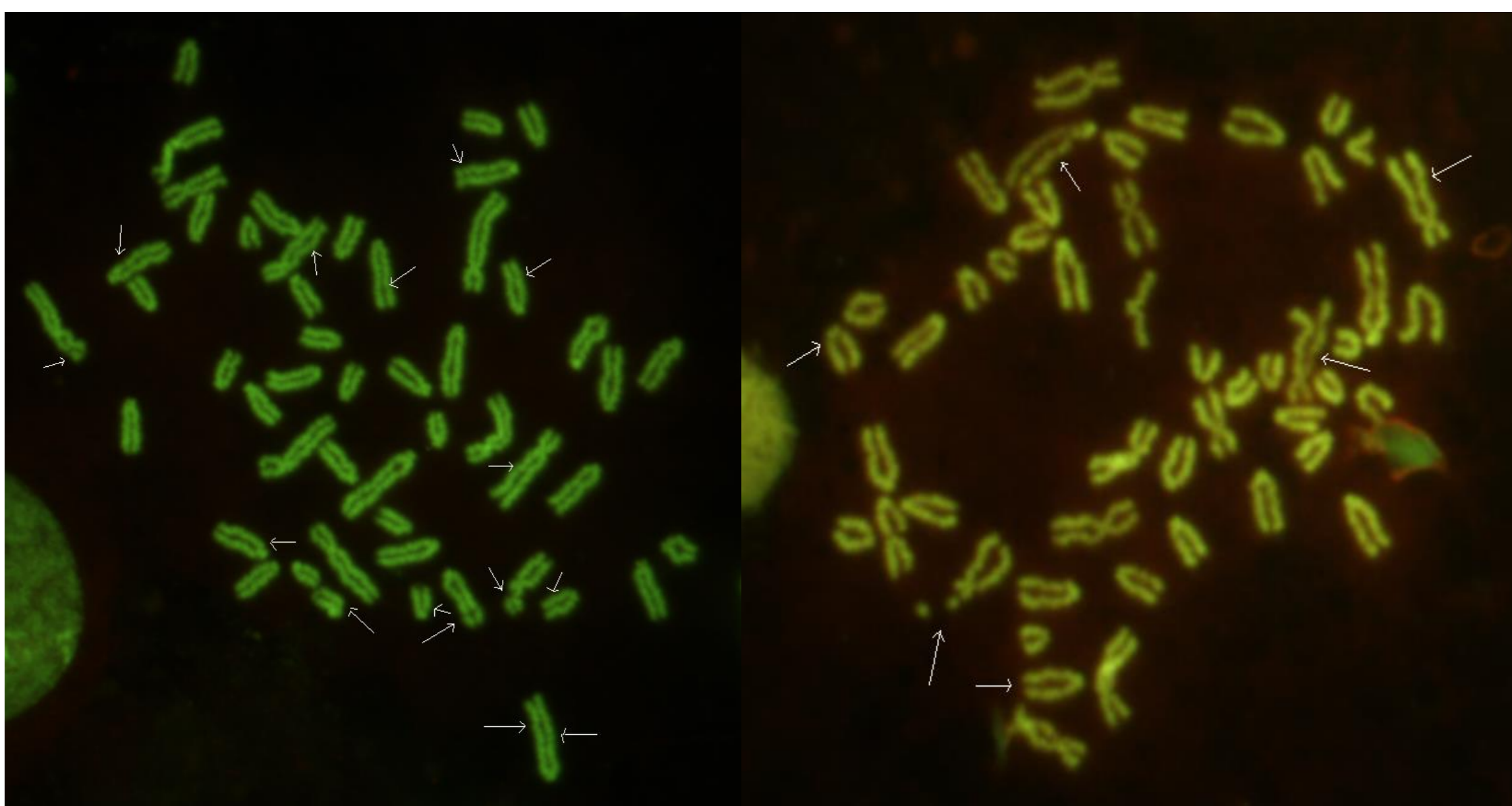


Figure 1. Metaphase spreads with many breakages and lost fragments indicated by arrows

### • Results and discussions

The cytogenetic investigation of the 287 buffalo females revealed normal karyotype,  $2n=50,XX$  for 240 females. We identified 46 cases of chromosomal instability represented by a large number of mono- and bi-chromatidic breakages on autosomes and heterosomes, loss of chromosome fragments and gaps (fig.1). Although the carriers have had a normal phenotype, the analysis of their reproductive activity revealed a degradation of the reproductive performances characterized by repeated inseminations, lack of oestrus and loss of pregnancy.

Our investigation continued through SCEs-test and for animals with many chromosomal breakages the number of sister chromatid exchanges (SCEs) was very high (11-23 SCEs/cell) compared to the normal animal (fig.2). These results suggest that the chromosomal fragility identified at the females with reproductive disturbances are characterized by a high rate of SCEs and could be related with the presence of different environmental toxic agents.

It was also identified a *Turner's syndrome* ( $2n = 49,X0$ ) in the case of a buffalo females with prominent withers, tight pelvis and reproductive disturbances.

### • Introduction

Cytogenetic investigation is a very important tool of evaluating the genetic health and fertility of farm animals. As well as the reproductive performance is the most important characteristic of the domestic animals, during the last years, a longstanding interest was dedicated to cytogenetic investigations in *River buffalo* females.

The presence, localization and frequencies of breakages and achromatic gaps could be used for testing chromosomal instability and for determination of their linkage with physiological and economic traits.

This work presents our observations concerning the role of chromosomal abnormalities as causes of reproductive failure in subfertile buffalo females.

### • Material and method

A total number of 287 Romanian buffalo females belonging to the R&D Station for Buffalo, Sercaia have been karyotyped by using peripheral blood lymphocytes culture for about 72 hours at  $38,5^{\circ}C$  in Minimal Essential Medium (Sigma) supplemented with 15 per cent fetal bovine serum (Sigma) and Concanavalin A as mitogen.

Two types of cell cultures were performed: without (normal cultures) and with addition of 5-bromodeoxyuridine (BrdU) during the last two cell cycles for the SCEs test.

Slides from both cultures were stained with acridine orange. At least 30 metaphase plates per animal were studied under a fluorescence Aristoplan Leitz microscope, captured with a Photometrics Cool Snap camera, transferred on PC and processed by a specific image software.

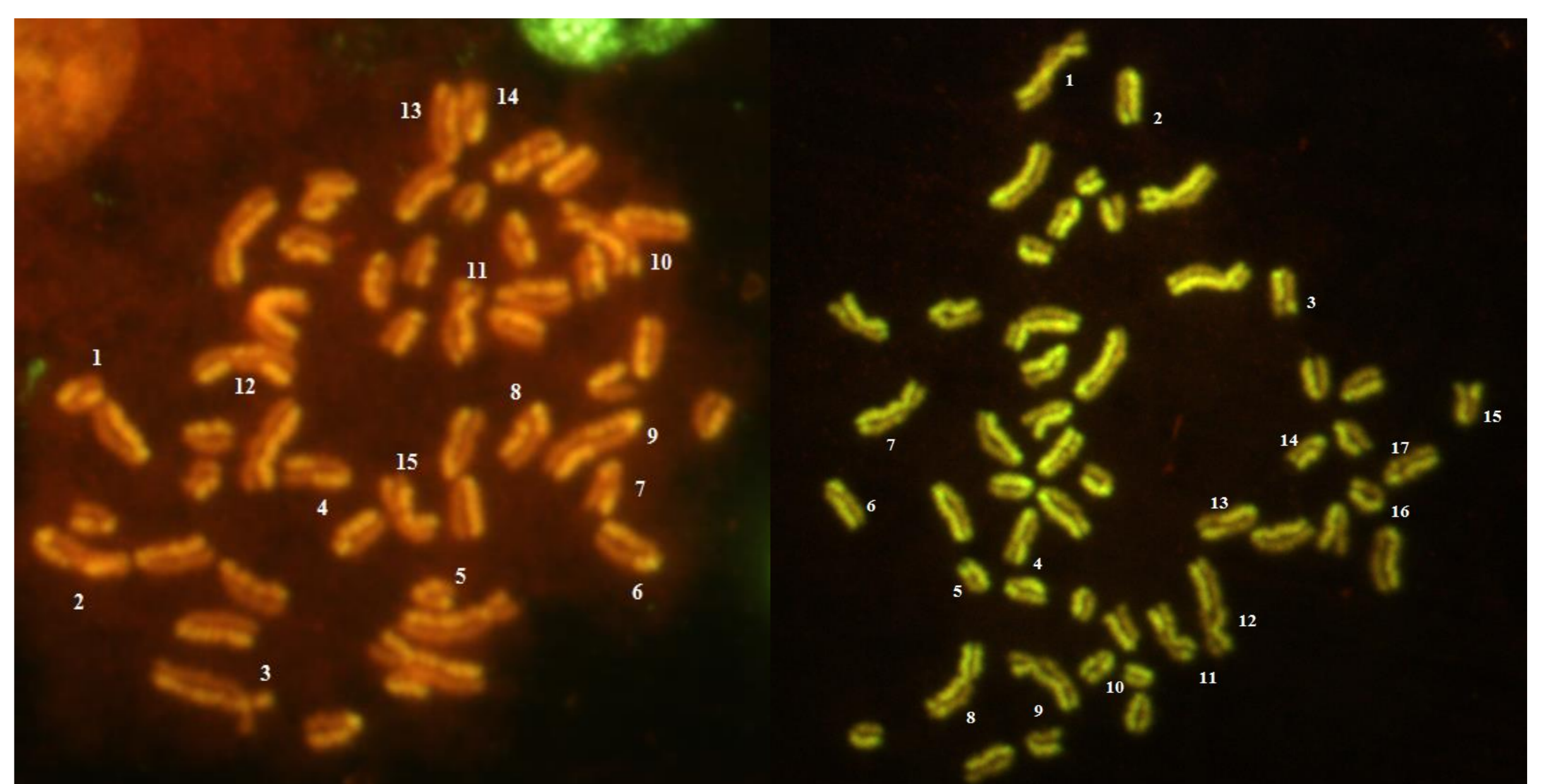


Figure 2. Metaphase spreads with SCEs indicated by numbers

### • Conclusion

According to these results the identified chromosomal abnormalities, demonstrated once again their role in the etiology of different levels of infertility.

**Acknowledgement:** Grant of the Ministry of Agriculture and Rural Development, project number ADER 8.1.4/2023.