ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. XXIX (3) SECTIO EE 2011

Department of Pig Breeding and Production Technology Departament of Animal Cytogenetics and Molecular Genetics, National Institute of Animal Production,
Krakowska 1, 32-083 Balice/Kraków, e-mail: bczech@izoo.krakow.pl
[2]Department of Pig Breeding and Production Technology, University of Life Sciences in Lublin,
Akademicka 13, 20-950 Lublin,
e-mail: marek.babicz@up.lublin.pl

BARBARA REJDUCH[1], ANNA KOZUBSKA-SOBOCIŃSKA[1],
BARBARA DANIELAK-CZECH[1], MAREK BABICZ[2]

Semen quality evaluation of young bulls carrying leukocyte chimerism 60,XX /60,XY

Ocena jakości nasienia młodych buhajów z chimeryzmem leukocytarnym 60,XX /60,XY

Summary. After cytogenetic analysis, 15 bulls were selected as carriers of 60,XX/60,XY chimerism to evaluate the influence of this chromosomal abnormality on reproductive performance. The semen analysis included the following parameters: volume of ejaculate (mL), motility of spermatozoa (%) and sperm concentration/mL × 10^6. The comparison of the experimental group with control (bulls with normal karyotype) showed significant differences with regard to the volume of ejaculate and highly significant differences with regard to the motility of spermatozoa and sperm concentration. These parameters were lower in the group of bull-carriers. Sperm-FISH technique made it possible to determine similar proportions between spermatozoa with X and Y heterosomes in bulls with karyotype 60, XX/60, XY (ranging from 45% : 55% to 57% : 43%).

Key words: leukocyte chimerism, bull, fertility, semen analysis, sperm-FISH

INTRODUCTION

Leukocyte chimerism 60,XX/60,XY is a frequent syndrome in heterosexual cattle twins. It is known that 89.5–93.6% of the female partners in dizygotic twin cattle are infertile and referred to as freemartins [Miyake *et al*. 1990, Rejduch 2001]. However, in the case of bull-carriers of leukocyte chimerism evidence of an influence of fertility is not conclusive [Świtoński *et al*. 1991, Rejduch *et al*. 1998]. Several authors have

claimed that such bulls demonstrated lower fertility [Stafford 1972, Cribiu and Popescu 1982, Świtoński *et al*. 1991, Rejduch *et al*. 2000], but others did not observe any abnormalities [Gustavsson 1977, Jaszczak et *al*. 1988, Kovacs and Karakas 1997, Rejduch 2001].

The aim of this paper is to find out if the bulls carrying the 60,XX/60,XY chimerism can be used in A.I. Stations.

MATERIAL AND METHODS

In present study ejaculate samples teken from fiftheen bulls of the Polish Red, Simental, Polish Holstein-Friesian, Red and White breeds aged 12–15 months originating from heterosexual twins, which were cytogentically diagnosed as the leukocyte chimerism 60,XX/60,XY carriers, were used. The control group included the same number of bulls from single-way pregnancies, of the same age. Blood samples for routine lymphocyte culture method were taken from animals under study and the karyotype analyses were carried out under light microscope using a conventionally stained preparations.

The following semen parameters were analysed: volume of ejaculate (mL), motility of spermatozoa (%), sperm concentration/ml $\times 10^6$ and spermatozoa morphology (% of major and minor defects). The samples were analysed using all the parameters mentioned above and average values for each bull were calculated (twelve samples per animal). One-way analysis of variance was applied to compare three parameters: volume of ejaculate, motile spermatozoa and sperm concentration, between two groups of young bulls: 15 animals from heterosexual twins and 15 control bulls.

To identify the hetrosomes in bull spermatozoa, FISH was applied following the procedures of Pinkel *et al*. [1986] and Solinas-Toldo *et al*. [1995] using bovine-specific X and Y painting probes. The X probe was amplified and labeled with DOP-PCR using the bio-16-dUTP nucleotide [Goldammer *et al*. 1996]. The Y probe was amplified by PCR as described by Guan *et al*. [1994] and PCR labelled with digoxigenin-11-dUTP (Boehringer Mannheim, Germany) according to the manufacturer's instruction. In each bull with leukocyte chimerism 60,XX/60,XY 500 spermatozoa were evaluated.

RESULTS AND DISCUSSION

Cytogenetic analysis of 15 bulls from dizygotic twins, showed the 60,XX/60,XY chimerism. Proportion of XY cell line ranged from 8 to 97% (Tab. 1). Amoung them three animals were culled because of very low growth rate (one bull) and poor sexual behaviour (two bulls).

Results of ejaculate analysis are presented in Table 1. The production of sperm ejaculates in a bulls with XX/XY chimmerism (calculated on the basis of 12 ejaculates) ranged from 1.16 mld till 8.780 mld (mean 3.23 mld). The percentage of major morphological deffects reached even 29.20 in one XX/XY chimeric bull (the mean for group of chimeric bulls – 8.17) and the minor deffects 62.61 in the same bull (the mean – 17.88).

The percentage of motile spermatozoa showed in Table 2 ranged the values from 23.5 to 73.4 and the sperm concentration from 289×10^6 to 1510×10^6.

Table 1. Production and morphology of sperm produced by XX/XY chimaeric bulls
Tabela 1. Produkcja i morfologia nasienia produkowanego przez buhaje z chimeryzmem XX/XY

Bull Buhaj	XY cell line Linia komórkowa XY (%)	Production of sperm (average of 12 ejaculates) (mld) Produkcja nasienia (średnia z 12 ejakulatów) (mld)	% of spermatozoa with morphological defects % plemników z wadami morfologicznymi	
			major główne	minor podrzędne
1.	95	4.27	3.70	3.77
2.	79	3.64	4.45	9.93
3.	40	8.78	3.74	11.22
4.	92	4.19	3.37	5.82
5.	52	1.16	3.86	9.06
6.	97	1.52	29.20	62.61
7.	14	1.66	13.38	16.20
8.	15	3.93	3.55	7.80
9.	45	2.68	5.86	12.70
10.	85	3.97	4.10	15.70
11.	30	5.12	2.10	11.10
12.	26	1.83	6.40	18.00
13.	16	1.47	6.00	21.30
14.	8	1.68	21.60	36.42
15.	90	2.48	11.30	26.50
Average XX/XY n = 15 Średnia XX/XY n = 15	52.27	3.23	8.17	17.88
Average control n = 15 Średnia kontrolna n = 15	100	3.62	5.30	6.40

The results of one-way analysis of variance showed significant differences between the volume of ejaculate and highly significant differences between motility of spermatozoa and sperm concentration of chimeric bulls and the control group.

Leukocyte chimerism, classified as a special category of changes in bovine karyotype, is the most widespread anomaly in cattle. Cytogenetic analysis makes it possible to calculate the percentage of female (XX) and male cell lines (XY) in the blood of a given animal. The percentage of each cell line may ranged from 0 to 100 [DeGiovanni *et al.* 1975]. In this study the proportion of XX to XY line range from 8 to 97%.

However, several reports showed that bulls with XX/XY leucocyte chimerism to have lower reproduction parameters. The investigations of Cribiu and Popescu [1982] discussed the non-return ratio for twelve chimeric bulls. For six of them the value of this parameter was significantly decreased. The same results were obtained by DeGiovanni *et al*. [1975] and Stafford [1972]. Świtoński *et al*. [1991] and Rejduch *et al*. [1998] showed a decrease of the non-return ratio in three of six chimeric bulls in comparison with the corresponding mean values of A.I. Stations.

Quantitative analysis of semen parameters, carried out by Dunn *et al*. [1979] on ejaculates produced by twelve chimeric bulls showed that the motility and sperm concentration were significantly lower than those of control bulls. These authors also pointed out on changes in the morphology, motility and vitality of spermatozoa with acrosome defects. Moreover, histopathological studies indicated degeneration in the testicular area.

Poor semen quality of chimeric bulls was also reported by Stafford [1972], Bongso *et al*. [1981] and Świtoński *et al*. [1991].

The results of our investigations (Tab. 2) showed a reduction of some semen parameters in bulls born co-twin with freemartins.

Table 2. Characteristics of ejaculates produced by XX/XY chimeric bulls
Tabela 2. Charakterystyka ejakulatów produkowanych przez buhaje z chimeryzmem XX/XY

Bull Buhaj	Volume (ml) Objętość (ml)	Motile spermatozoa (%) Ruchliwość plemników (%)	Sperm concentration/ml ($\times 10^6$) Koncentracja plemników/ml ($\times 10^6$)
1.	4.86	65.0	879
2.	5.03	56.7	724
3.	5.85	73.4	1510
4.	4.88	68.3	858
5.	2.26	60.8	504
6.	1.31	23.5	398
7.	3.00	45.0	552
8.	3.95	64.0	971
9.	3.11	55.8	863
10.	4.77	61.7	832
11.	5.57	55.8	918
12.	3.03	45.0	606
13.	2.62	48.3	560
14.	2.36	41.7	289
15.	3.24	53.3	968
Average XX/XY n = 15 Średnia XX/XY n = 15	3.72*	54.85**	762**
Average control n = 15 Średnia kontrolna n = 15	4.39	70.1	1113

* significant differences ($P < 0.05$) – różnice istotne ($P < 0,05$)

** highly significant differences ($P < 0.01$) – różnice wysoko istotne ($P < 0,01$)

Jażdżewski [1976] showed that in two groups of 15-month-old bulls of the Polish native Black and White breed, kept in different conditions, the volume of ejaculate ranged from 3.72 to 4.00 ml, motile spermatozoa ranged from 62.9 to 66.8 percent and sperm concentratiom ranged from 1005 to 1136/ml × 10^6.

In our study on chimeric bulls, the corresponding parameters were as follows: volume of ejaculate – 1.31–5.85 mL (mean 3.72 mL), motility of spermatozoa – 23.5–73.4% (mean 55.85 %) and sperm concentration – 289–1510/ml × 10^6 (mean 762/ml × 10^6).

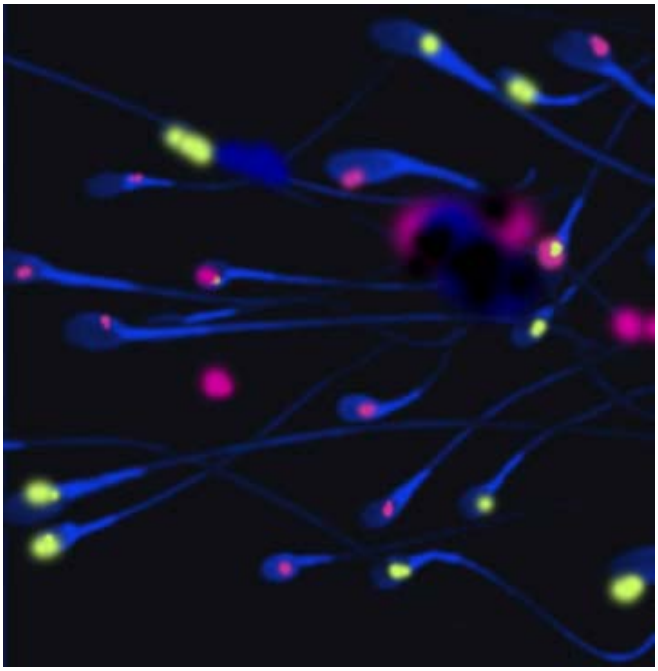

Figure 1. FISH technique. Fluorescent signals in bull spermatozoa
Rysunek 1. Technika FISH. Sygnały fluorescencyjne w plemnikach buhaja

The hybridization of bovine heterosome painting probes with genetic material found in bull semen revealed clear single fluorescent signals: yellow on sperm with X chromosome, and red-purple in cells with Y heterosome (Fig. 1). Based on analysis of hybridization signals evaluated in 500 spermatozoa of every animal and using the image analysis sytstem LUCIA-FISH, the proportion of sperm with X chromosome in the haploid set (45–55%), and the proportion of sperm with Y chromosome (57–43%) were determined, taking into account those spermatozoa for which no signals were recorded (0–4%). Simultaneous hybridization using two differently labeled probes performed in bull spermatozoa by Hassanane *et al*. [1999] made it possible to determine appox. 0.1% of disomic spermatozoa (0.067% XX, 0.029% YY and 0.029% XY) in the analysed sperm in addition to determining the X:Y ratio. The use of the FISH technique for identification of the heterosomes in the semen of Bovids species diagnosed with leukocyte chimerism

XX/XY provides new insights for the debate on uneven sex distribution among the offspring of males with chimerism [Lojda 1972, Rejduch 2001, Di Berardino *et al*. 2004, Kozubska-Sobocińska and Rejduch 2008].

CONCLUSIONS

Based on the results obtained, which characterise fertility of bulls carrying the XX/XY chimerism, we suggest that these animals should not be used in A.I. Stations.

REFERENCES

Bongso T.A., Jainudeen M.R., Lee J.Y.S., 1981. Testicular hypoplasia in a bull with XX/XY chimerism. Cornel Vet., 71, 376–382.

Cribiu E.P., Popescu C.P., 1982. Sur la frequence du chimerisme leucocytaire XX/XY parmi les taureaux d'insemination. 5th European Colloquium on Cytogenetic of Domestic Animals, Milano-Gargnano, Proceedings, 215–221.

DeGiovanni A., Popescu C.P., Succi G., 1975. Premiere etude cytogenetique dans un centre italien d'insemination artificielle. Ann. Genet. Sel. Anim., 7, 311–315.

Di Berardino D., Vozdova M., Kubickova S., Cernohorska H., Coppola G., Coppola G., Enne G., Rubes J., 2004. Sexing river buffalo (*Bubalus bubalis* L.), sheep (*Ovis aries* L.), goat (*Capra hircus* L.), and cattle spermatozoa by double color FISH using bovine (*Bos taurus* L.) X- and Y-painting probes. Mol. Reprod. Dev., 67, 108–115.

Dunn H.O., McEntee K., Hall C.E., Johnson R.M., Stone W.H., 1979. Cytogenetic and reproductive studies of bulls born co-twin to freemartins. J. Reprod. Fertil., 57, 21–30.

Goldammer T., Weikard R., Brunner R.M., Schwerin M., 1996. Generation of chromosome fragment specific DNA sequences by microdissection and DOP-PCR. Mamm. Genome, 7, 291–296.

Gustavsson I., 1977. Fertility of sires born as dizygotic twins and sex ratio in their progeny groups. Ann. Genet. Sel. Anim, 9, 531.

Guan X.Y., Meltzer P., Trent J., 1994. Rapid generation of whole chromosome painting probes (WCPs) by chromosome microdissection. Genomics, 22, 101–107.

Hassanane M., Kovacs A., Laurent P., Linblad K., Gustavsson I., 1999. Simultaneous detection of X- and Y- bearing bull spermatozoa by double colour fluorescence *in situ* hybridization. Hun. J. Anim. Prod., 48(1), 128.

Jaszczak K., Parada R., Boryczko Z., Romanowicz K., Wijas B., 1988. A cytogenetic analysis and reproductive ability of bull having karyotype with only XX sex chromosomes in the blood lymphocytes. Genet. Pol, 29, 368–375.

Jażdżewski J., 1976. Forming of sex functions and fertility of Black-White bulls at 10–15 months of age. Roczn. Nauk. Zoot, 6,111–151.

Kovacs A, Karakas P., 1997. Routine chromosome investigations on cattle. Streszczenie, V Kolokwium Cytogenetyczne, Balice, 4–5 listopada, 12–14.

Kozubska-Sobocińska A., Rejduch B., 2008. Identification of heterosomes in spermatozoa of rams with 54,XX/54,XY chimerism. Vet. Med. Czech., 53(5), 250–254.

Lojda L. 1972. Chromosomal chimerism of the sire as a cause of the shift in sex ratio in his offspring. Proc. 7th Int. Cong. Anim. Reprod., 2, 1110–1113.

Miyake Y-I., Murakami R-K., Kaneda Y., 1990. A single-born infertile heifer with sex chromosome chimerism (XX/XY). J. Facul. Agric., 19, 333–338.

Pinkel D., Gray J.W., Trask B., van den Engh G., Fuscoe J., Van Dekken H., 1986. Cytogenetic analysis by in situ hybridization with fluorescently labelled nucleic acid probes. [In:] Proceedings of Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor Laboratory, 1, 151–157.

Rejduch B., Słota E., Kozubska-Sobocińska A., Jażdżewski J., Danielak-Czech B., 1998. Semen characteristic of bulls – carriers of XX/XY leucocytic chimerism. Rocz. Nauk. Zoot., 25(4), 9–15.

Rejduch B., Słota E., Gustavsson I., 2000. 60,XY/60,XX chimerism in the germ cell line of mature bulls born in heterosexual twinning. Theriogenology, 54(4), 621–627.

Rejduch, B., 2001. Zjawisko chimeryzmu komórkowego u zwierząt z rodziny *Bovide*. Rocz. Nauk. Zoot., Rozpr. hab., 14, 1–61.

Solinas-Toldo S., Lengauer C., Fries R., 1995. Comparative genome map of human and cattle. Genomics, 27, 489–496.

Stafford M. J., 1972. The fertility of bulls born co-twin to heifers. Vet. Rec., 90, 146–148.

Świtoński M., Lechniak D., Landzwojczak D., 1991. Cytogenetic survey of bulls used in artificial insemination. Reproductive performance of XY/XX chimeric bulls. Genet. Pol., 32, 227–233.

This work was conducted as part of statutory activity of the NRIAP, project no. 3215.1.

Streszczenie. Na podstawie analizy cytogenetycznej wytypowano osobniki do grupy doświadczalnej liczącej 15 buhajów – nosicieli chimeryzmu 60,XX/60,XY w celu określenia wpływu tej nieprawidłowości kariotypu na parametry nasienia. Analiza nasienia obejmowała następujące parametry: objętość ejakulatu (ml), ruchliwość (%) i koncentracja plemników/ml × 10^6. Porównanie grupy doświadczalnej z kontrolną (buhaje o prawidłowym kariotypie) wykazało istotne różnice dotyczące objętości ejakulatu oraz wysoko istotne różnice dotyczące ruchliwości i koncentracji plemników. Buhaje z chimeryzmem leukocytarnym charakteryzowały się obniżonymi parametrami nasienia. Technika sperm-FISH umożliwiła określenie podobnego udziału frakcji plemników z chromosomem X i Y u buhajów o kariotypie 60, XX/60, XY (w zakresie od 45% : 55% do 57% : 43%).

Słowa kluczowe: chimeryzm leukocytarny**,** buhaj, płodność, analiza nasienia, sperm-FISH