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Haematopoietic chimerism expressivity in bovine heterosexual twins

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Relatively increased frequency of twinned multifoetus births is characteristic for highly productive breeds. However, researchers consider twins undesirable because the frequency of cattle haematopoietic chimerism is sufficiently high in heterosexual twins and female calves co-twined to bull-calves are sterile freemartins (Esteves *et al.* 2012). Nevertheless, the issues related to chimerism regularity and expressivity and some features of the blood cell populations available in the animals concerned remain insufficiently examined till now. The issues gained particular importance when it comes to the prevalence of embryo transfers in which it is common enough to transplant some embryos (Hirayama *et al.* 2013) to recipient-cows to enhance the probability of giving birth to living calves. In artificial twinning, the very procedure of transplantation and characteristic features of recipients can have effects on hematologic relationships of embryos. However, there are no reasons to assume that the artificial twinning resulting from the transplantation introduces radically new elements of the relationships between embryos, which do not occur under natural conditions. The artificial twinning allows the study of haematopoietic chimerism variants relevant to natural variants and besides, to look for the characteristics of cell populations associated with the phenomenon. Therefore, the paper examines haematopoietic chimerism expressivity in the bovine twins labored both the birth after AI and after embryo transfer.

Black and White Holstein cattle from the breeding-farms of Novosibirsk and Kemerovo regions were used for cytogenetic examinations of 110 co-twins (aged 3–18 months) born after artificial insemination. They were phenotypically healthy, but in some cases, different developmental anomalies of the reproductive system were identified in the animals. Seven freemartin heifers were slaughtered and their reproductive organs were removed and examined morphologically. Simmental animals housed under the conditions of Prilutsky breeding-farm were also involved in the examination. The following animals were examined: an embryo donor-cow (aged 8 years) and four pairs of her offsprings (aged 6 months) grown from the embryos transferred to different recipients. The blood cells cultivation and production of metaphase preparations were done (Halnan 1977). Neither colchicine nor colcemidum were added to avoid breaking down interchromosomal associations by analogy with Robertsonian translocations. The end of the incubation time (48 h) and harvesting of chromosomes coincided. Statistical validity for the difference in the occurring incidence of cytogenetic anomalies among the groups of animals was assessed with z-test.

One of the clones containing either XY or XX chromosome sets predominated in 79% of the cases with Black and White Holstein twins examined, both in bull- and female-calves. The following regulation was observed in 5 sibling pairs, the female-calves had the highest percentage of cells with chromosomal status XX, the bull-calves accordingly, had XY.

One pair of the 5 sets of twins was revealed to have the bull-calf possessing 60, XX set, and the female-calf – 60, XY, in all the metaphases examined. In the other pair, it was the XX set that was disclosed in the cells in the male calf whereas the female was found out to have 2 clones of cells in the ratio 52XX:48XY.

On average, the part of typical cells in male co-twins is identified to exceed migrant cells by 2%, in female by 10.8% (Table 1).

Proliferative activity of the XX cells may be higher than that of XY cells. This is also evidenced by the fact that, the level of chimerism occured in male calves 5.8 times higher than female calves. It is known that in the embryonic period

Table 1. The ratio of XX and XY cells in the twins examined

Parameters	Male	Female
	n=43	n=67
XX cells, %	49.0±0.05	55.4±0.04
mean±SD	0 - 100	0 - 100
lim		
XY cells, %	51.0±0.05	44.6±0.04
mean±SD	0 - 100	0 - 100
lim		

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of heterosexual twin's development, the secretion of male hormones begins earlier than that of female ones, which leads to a deviation in female reproductive organs development (Jimenez *et al.* 2013, Remnant *et al.* 2014, Biswas *et al.* 2014). Generally heifers become infertile, which is caused by underdeveloped or disorders of genital organs (Petrizzi *et al.* 2002). We monitored the development of fertility-related traits in some heifers with haematopoietic chimerism. Upon reaching the breeding age, and after a series of unproductive inseminations, heifers were slaughtered and the anatomical and morphological description of their reproductive organs was performed (Table 2).

Migrant-cells XY predominance in heifer's blood can be associated with the total absence of reproductive organs. Considerable disorders in the reproductive system are marked even with a comparatively small per cent of bull-derived cells present (heifer 1420). In the examined bull-calves from unlike sexed twins, the percentage of metaphases with chromosomal set XX varied from 32.3 to 88.0%. It is marked that the clones of two bulls with chromosomal set XX predominated. The data obtained testify to the wide individual variability of haematopoietic chimerism expressivity in females.

When studying metaphases of cow-donor of embryos, we found out the phenomenon of increased fragility in one of the X-chromosomes. References describe the phenomenon concerned in Holstein-Friesian cattle (Llambi and Postiglioni 1994) and freemartins (Peretti *et al.* 2008). In our studies, the phenomenon was followed by heteromorphism for the length of homologous X-chromosome in Holstein cows. Heteromorphism of sex chromosomes concomitant with increased fragility in one of them was found in some offspring of the cow-donor examined, particularly in heterosexual heifer-twins. The phenomenon available enabled us to estimate haematopoietic chimerism expressivity in this pair, since it was clarified that metaphase plates with heteromorphic chromosomes X are appreciably different in the frequency of their occurrence (Table 3).

Table 2. Reproductive system anomalies in the freemartin heifers with the different ratio of cell clones (XX: XY)

Heifer number	Cell clone				Reproductive system anomalies
	XX		XY		
	n	%	n	%	
1685	9	11.8	67	88.2	Hyperemic vagina, no folding. Rudimentary clitoris
382	7	43.8	9	56.2	Gartner ducts underdeveloped, ended in two diverticula
1853	43	24.1	135	75.9	Lack of uterus, ovaries, ovarian tube,
8250	38	38.0	62	62.0	Shortened vagina. One ovary, completely filled with yellow body. Lack of ovarian tube and uterus
852	5	4.4	108	95.6	Constricted and enlarged vagina. Reduced clitoris. Underdeveloped uterus. Lack of ovaries, ovarian tube
4549	37	37	63	63	Hyperemic vagina. Well-expressed clitoris. Uterus bifurcation and poorly developed neck of uterus available. Ovaries hypoplasia
1420	63	85.2	11	14.8	Vagina elongated. Lack of clitoris, uterus. Adhesion of the walls in *posterior vaginal fornix.* One ovary available, filled with connective tissue

Table 3. Frequency of different karyotypic anomalies (%) in donor-cow embryos and in the animals derived from pairwise embryo transfer to different recipients (HM – metaphases with heteromorphic X-chromosomes)

Animals	Investigated cells	Type of cells				
		Diploid	Aneuploid	Polyploid	Interchromosomal associations of Robertsonian translocations type	XX, XY or HM (n)
Cow-donor	115	72	12	16	3	—
Siblings						
Female No. 1004	115	58	35	7	7	XY (60)
Male No. 1003	69	71	24	6	11	XY (94)
Female No. 1006	45	47	16	37	0	XX (89)
Male No. 1005	80	63	37	0	21	XX (82)
Female No. 1011	52	62	21	21	7	HM(75)
Female No. 1010	21	72	22	5	11	HM (48)
Female No. 1014	26	31	50	19	0	XX (79)
Male No. 1013	26	48	44	8	15	XY (100)

In the case of like sexed twins, the both heifers were estimated for the frequency metaphases with heteromorphic X-chromosomes and metaphases in which X-chromosomes did not differ from each other. The first variant of metaphases is designated as HM (metaphases with heteromorphism for XX chromosomes).

The data presented in Table 3 unambiguously testify to the wide range of variability for the expressivity and direction of haematopoietic chimerism even in the offspring of the same parents. Thus, almost all the cells of the bulls in twins No. 1003 and No. 1004 carried XY chromosomes, but regarding the heifers, the cells of both sexes occurred and metaphases XY also dominated. Contrary is the case in the second pair, cells XX dominated in the both animals. Eventually, one case with the third pair of like sexed twins witnessed predominating cells with heteromorphism of sex chromosomes, the other case with the same pair observed both variants of the cells occurring with almost the same frequency. It was in a female in the pair of twins No. 1014 and No. 1013 that chimerism was identified, the one being poorly expressed in the female and totally absent in the male.

Thus, the data obtained testify to the wide range of haematopoietic chimerism expressivity variation in cattle twins (from 0 to 82 % of the cells from one individual can be represented by the cells from the other twin) and the expressivity does not depend upon sex (male cells dominated in 2 pairs of twins, in 1 pair of females; haematopoietic chimerism is also observed in the like-sexed pair).

The wide variability for haematopoietic chimerism direction and expressivity could be, in principle, explained by the effect of at least two causes: (i) transfer procedure and characteristic features of embryo recipient on the processes of placental anastomosis formation between them; (ii) heterogeneity of blood stem cells for the capacity to migrate into the blood stream of the other co-twin and for the 'from an individual to an individual' variability of stem cells share which possess the capacity concerned.

In the present paper we had no chance to assess the effect of the same recipients on the expressivity and direction of chimerism of the embryo pairs transferred to them. However, our data has allowed the comparison of some characteristics of immigrant cells which are the offspring of a haematopoietic stem cell that run into the other organism during embryogenesis with general characteristics of cell populations, which are the offspring of host stem cells.

The comparison for a series of characteristics of karyotypic variability of blood cells in the animals examined (Table 3) showed that twins can, indeed, differ from each other appreciably for these indexes regardless the fact that their blood cells have a common origin. The pair of twins No. 1006 and No. 1005 is the most obvious example in this respect. Blood cells of the animals are mainly presented by two sex chromosomes XX (82 and 89%), i.e., they are derived from the general population of heifer's haematopoietic stem cells. However, the frequency of occurring different karyotypic anomalies in these twins radically differ from each other. They are significant different for the share of aneuploid and polyploids cells, and metaphases with interchromosomes fusions (the type of Robertsonian translocations). The example is an illustrative evidence that, in general, *in vivo* very high is the potential of the same origin cells to form cell clones with different cytogenetic anomalies, the one is, apparently, not less than in cell cultures (Luik and Kochneva 2008) and it is largely scanty when it comes to the conditions for defective cells selection in multi-celled organism.

We would like to draw your attention to the following: the share of aneuploid cells was higher in the two pairs of twins (No. 1004, 1003 and 1006, 1005, Table 3), and it was higher in the animals which haematopoietic chimerism was the most expressed. On average, provided that the incidence of aneuploidy is calculated separately for each pair of animal twins with predominating self-cells or genetically alien, the following will be observed. In the animals with predominating migrant-cells the average incidence of aneuploidy was 36.0±5.7%, but in the partners with predominating self-cells it was 21.0±6.8%. This means that the differences are reproduced from pair to pair, however, as a total, they are on the verge of significance due to the variability of the trait from the pair to pair in twins.

A higher percentage of aneuploid cells in freemartins compared with control group of cattle were established by Peretti *et al.* (2008). There are no expressed differences involved between the groups for the share of polyploidy cells and metaphases with inter-chromosomal associations for the type of Robertsonian translocations (RBT) some pairs having them evident though.

Provided that the incidence of the associations mentioned is calculated directly for each animal individually in self-cells (sex chromosomes which correspond to the animal sex) and in the cells, which are the offspring of haematopoietic stem cells of the partner (immigrant cells), the following differences will be obtained, the frequency of metaphases with RBT averaged 6±3% for an animal in self-cells and the one did 16±5% in immigrant cells. This means that there is a tendency observed to increased aneuploidy in the animals with expressed haematopoietic chimerism, but in the offspring migrated to the alien organism of haematopoietic stem cells there is a tendency marked to increased frequency of metaphases with inter-chromosome associations for the type of Robertsonian translocations. It should be noted that among the aneuploids examined in this case (43–66 chromosomes) hypodiploid cells predominated, which emergence may relate much more to the decreased stability of plasmatic membranes and chromosomes loss when making preparations than to real disorders in chromosomes segregation. It may be expected that the increased share of aneuploids of the type in immigrant cells testify to their decreased plasmatic

membranes stability. Tetraploid was found in one of the examined bull-calves (No. 1003) wherein the combination of sex chromosomes XXXY was observed. Thus, this tetraploid emerged resulting from the fusion of 2 somatic cells of different genetic origin.

It is common assumption that polyploid cells in mammalian blood are the result of disorders in mitosis and cell-cell fusions are typical of cell cultures, tumor cells and induced by ionizing radiation and certain viruses. The tetraploid identified enables us to state that blood cells fusion can also take place in phenotypically healthy animals with haematopoietic chimerism.

A total of the data obtained testify to possibly available specific structural-functional traits of haematopoietic stem cells which make them capable of immigrating into the blood-vascular system of co-twins in embryogenesis. Variability of their share may underlie the differences in haematopoietic chimerism expressivity and direction which are observed even in the offspring of the same parents. The share of the cells involved may be expected to be, to a certain extent, genetically determined. In that case, the trait may become the object of breeding and further on lead to the formation of multifoetus families in cattle, among which haematopoietic chimerism is either absent in twins or realized only in bull-calves.

SUMMARY

The aim of the experiment was to study the haematopoietic chimerism in bovine heterosexual twins conceived through artificial insemination and post-embryo transfer. Both animal groups were revealed to have a wide individual range of variability for the expressivity of chimerism that varied from 0 to 96% of cells with the chromosomes of an opposite sex. The study also revealed the tendency towards increased frequency of cytogenetic anomalies in immigrant cells.

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