Leucocyte and erythrocyte chimerism in heterosexual bovine quadruplets

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Since Owen's (1945) discovery of erythrocyte chimerism in heterosexual bovine twins, several investigators reported the co-existence of male and female cells among the leucocytes of these cattle (OHNO *et al.*, 1962; FECHHEIMER *et al.*, 1963; GOODFELLOW *et al.*, 1965; KANAGAWA *et al.*, 1965 b; BASRUR and STOLTZ, 1966). However, a majority of these reports are limited to chimerism in either erythrocytes (OWEN, 1945; RENDEL *et al.*, 1962; STONE and CRAGLE, 1964) or leucocytes (KANAGAWA *et al.*, 1965 b; MCFEELY *et al.*, 1967; BASRUR and KANAGAWA, 1969), and in a few instances in germ cells (OHNO *et al.*, 1962; OHNO and GROPP, 1965; KANAGAWA *et al.*, 1965 b; TEPLITZ *et al.*, 1967). The present report concerns a comparative analysis of erythrocyte antigens and cytological sex of cultured leucocytes in heterosexual quadruplets.

Materials and Methods

A 10.5-year-old Holstein cow gave premature birth to a litter of four, three freemartins and one male. They were calved about 19 days earlier than expected, but were all healthy (Service date of August 25, 1955. Birth date of May 12, 1966). Their sire and dam were registered as Holstein Herd Book Registry-No. 35001, and as Holstein Supplementary Registry-No. KFS 571594, respectively.

Blood samples for the examination of leucocytes and erythrocytes were obtained from the jugular vein when the quadruplets were 13 days. Chromosome study was carried out on leucocyte cultures according to the method described previously (KANAGAWA *et al.*, 1965 b). Blood type analysis was based on the method of standard hemolytic test partly modified by KOSAKA *et al.*, (1967).

Three of the quadruplets were sacrificed at the age of one month, and the gross morphology and the microscopic features of the reproductive organs were observed. The remaining calf is still under observation.

Results

Table 1 presents the chromosome findings on cultured leucocytes and the pattern of erythrocyte antigens in quadruplets. Chimerism for male and female cells was noted in all 4 calves. The ratio of male and female cells are seemed to be approximately similar in all calves irrespective to their phenotypic sex, the XX cells being the highest, ranging from 93.2 to 95.7 %.

On the other hand, erythrocytes appeared to consist of 4 different genotype, $A_1/-$; $A_2/-$; A_1/A_2 ; $-/-$ in A system and $GY_2E'_2/Y_1$; $GY_2E'_2/E'_3O'_2Y'_1$; $O_3J'K'O'_2/Y_1$; $O_3J'K'O'_2/E'_3O'_2Y'_1$ in B, showing a state of quadruple chimerism. In other systems, reaction was seen L, S-U and Z, whereas tests for J were negative in all calves. Although the analysis of red cell antigens immunogenetically insufficient in C, C_1 and W_1 antigen showed partial hemolysis.

Table 1. Chimerism in leukocytes and erythrocytes of bovine quadruplet

Sex	Sex chromosome		Blood type system									
	XX(%)	XY(%)	A	B	C	F-V	J	L	S-U	Z	R'-S	
Female # 1	130 (95.6)	6 (4.4)	[	A ₁ /—	GY ₂ E'₂/Y₁	W₁/CX₁	F/F	—/—	L/—	H'/—	Z/—	S'/S'
Female # 2	120 (94.5)	7 (5.5)		A₂/—	GY ₂ E'₂/E'₃O'₂Y'₁	CX₁/—	F/F	—/—	L/—	H'/—	—/—	S'/S'
Male # 3	124 (93.2)	9 (6.8)		A₁/A₂	O₃J'K'O'₂/Y₁	X₂/W₁	F/F	—/—	L/—	—/—	—/—	S'/S'
Female # 4	200 (95.7)	9 (4.3)		—/—	E'₃O'₂Y'₁/O₃J'K'O'₂	X₂/—	F/F	—/—	—/—	—/—	—/—	S'/S'
Sire	*	*		A₂/—	GY₂E'₂/O₃J'K'O'₂	W₁/—	F/F	—/—	—/—	H'/—	Z/—	S'/S'
Dam	*	*		A₁/—	Y₁/E'₃O₂Y'₁	CX₁/X₂	F/F	J ^{CS} /—	L/—	H'/—	—/—	S'/S'

* Chromosomes were not examined

[] Quadruple chimerism

Macroscopical appearances of the sexual organs of 2 freemartins are shown in figures 2 and 3. In both cases, several typical morphological characteristics of freemartin were observed: absence of the oviduct and cervix, the hypoplastic uterus and anterior vagina, the overdeveloped clitoris, the presence of the seminal vesicle-like structures, rudimentary structures of the Wolffian ducts and the well-developed ovarian blood vessel plexuses.

Histologically, the bilateral gonads in both cases showed immature ovary of a normal heifer but failed to show structures resembling seminiferous tubules. However, in both cases, at the surrounding portion of the rete ovarii, there existed a mass of small tubes somewhat resembling immature seminiferous tubules. In the uterine horns and seminal vesicle-like structures in both freemartins, undeveloped gland-like structures were observed in the tunica propria. The rudimentary Wolffian ducts had a central cavity and the surrounding portion containing small gland-like structures scattered here and there.

Discussion

Members of the same litter generally show a similar ratio of mixing of leucocytes and erythrocytes in cattle (BASRUR and KANAGAWA, 1969). The pattern of chimerism in leucocytes appears to be similar during the development despite the difference in the cell type analysed. The occurrence of chimerisms and the absence of fluctuation of sex ratio among the cells might be either due to the difference in the stage of development at which the shift of primordial germ cells (OHNO and GROPP, 1965) and primordial hematopoietic cell (OWEN, 1945; OWEN *et al.*, 1946) took place. Alternatively, it may be attributable to the quantitative relationship of transplanted cells, or due to the early selection of donor or host cells in the recipient gonad or blood forming tissue. The answer will not be obtained until a successful analysis of chromosomes in the gonads of chimeric cattle is accomplished. KANAGAWA *et al.*, (1965 a) and KANAGAWA and BASRUR (1968), have reported that early diagnosis of bovine freemartinism is possible through the leucocyte culture technique which relies on the morphology of sex chromosomes. The chimeric ratios in this study were remarkably parallel with each other in the

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members of each set of litter mates. Histologic characteristics of the gonads of 2 freemartins in quadruplets revealed resemblance to immature ovaries and the rate of the percentages of XX cells in these animals were high. The degree of histologic deviation in freemartin gonad does seem to depend upon the ratio of male cells in freemartins (HERSCHLER and FECHHEIMER, 1967). HERSCHLER and FECHHEIMER (1967) have reported that the primary etiological factor of gonadal modification in freemartin is probably the Y chromosome-bearing cells at the time of differentiation. The mechanisms involved in the selection or rejection of cell types, which interchanged during embryonic growth and "homing" in different tissues of the hosts, are at present not really known.

Summary

Chromosome analysis from cultured leucocytes and blood type analysis from erythrocytes were carried out in heterosexual bovine quadruplets. Chimerism for male and female cells in the cultured leucocytes of quadruplets were observed. The ratio of male and female cells being approximately similar in members of sets. Also, erythrocytes appeared to consist of 4 different genotypes in A and B system, showing a state of quadruple chimerism in quadruplets.

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牛異性四仔における白血球と赤血球のキメラ

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異性双仔間の胎生期における血管吻合の結果生じる2・3の細胞のキメラ現象は、赤血球においては表面の血液型抗原を支配する遺伝子レベルで、白血球においては染色体の構成差(♂2 A-XY, ♀2 A-XX)から、それぞれ分析されフリーマーチンの早期診断に利用されるようになっている。しかし、双仔以上の多胎については、白血球と赤血球の関連・キメラの形・キメラ体の繁殖性などにおいて不明確な点が多い。

本実験は雄1例・フリーマーチン3例からなる異性四仔について、白血球と赤血球のキメラの比較・キメラの構造および程度・雌子のフリーマーチン化の程度などの分析を行なったものである。白血球の培養は37°C・72～96時間・コルヒチン処理・水処理・カルノア液固定後、細胞浮遊液の空気乾燥による染色体標本の作製を行ない、ギムザ液染色を施し個々の細胞の性染色体を観察した。赤血球はA・B・C・F・V・J・L・S・U・Z・R'-S'の9 Blood Type Systemの遺伝子分析を行なったものである。

その結果、

1. 白血球においては各仔共に100個以上の細胞の性

染色体を分析した結果、雌型細胞(2 A-XX)を百分率で表示する場合、#1(♀); 95.6・#2(♀); 94.5・#3(♂); 93.2・#4(♀); 95.7を示す2 A-XXと雄型細胞(2 A-XY)のキメラがみられ、四仔の雌雄に関係なくほとんど同率に分布していることが明らかにされた。

2. 血液型反応においては各血球共に部分溶血を同程度に示しており、血液型の四重キメラを推定し得る。すなわち、A Systemでは $A_1/-+A_2/-+A_1/A_2+/-$, B Systemでは $GY_2E'_2/Y_1+GY_2E'_2/E'_3O'_2Y'_1+O_3J'K'O'_2/Y_1+O_3J'K'O'_2/E'_3O'_2Y'_1$ を推定させるキメラ特有の溶血がみられた。その他のL・S・U・ZおよびC Systemにおいてもキメラが証明された。

3. 四仔の中#4を除き屠殺解体されたが、異性双仔のフリーマーチンと同じく、幼若精嚢の出現、子宮体部の欠除、ピーナッツ大に退化した子宮角・痕跡的存在の卵巣部が特徴的変状であり、組織学的には子宮角の腺は著しく乏しく、性腺においては幼若卵巣の所見がみられ部分的に幼若精細管類似の小管腔と周囲に蔓状静脈叢類似のよく発達した血管叢がみられた。雄仔は正常の生殖器、副生殖器を有していることが確認された。

Plate

Fig. 1. Frontal views of a set of heterosexual quadruplets. Nos. 1, 2 and 4 are freemartins, and No. 3 is a male.

Figs. 2 and 3.

A general appearance of reproductive organs from freemartins (Fig. 2: case #1 and Fig. 3: case #2). Arrows indicate the sites of gonads (scale: cm).

Figs. 4 and 5.

Metaphase chromosomes from leucocyte cultures of a freemartin (case #1), showing XX/XY chimerism. Giemsa stain. X 2,000. Fig. 4: A metaphase plate showing the female bovine complement including two submetacentric X chromosomes. Arrows indicate sex chromosomes. Fig. 5: The same, showing the male bovine complement. Note one large submetacentric X chromosome and a small metacentric Y chromosome. Arrows show X and Y, respectively. The bovine autosomes are all acrocentric.

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