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Stanisław FEDYK & Małgorzata KRASIŃSKA

Studies on the Spermatogenesis in European Bison and Domestic Cattle Hybrids

[With 3 Tables, 1 Text—Fig. & Plates IV—V]

The investigations are described on the spermatogenesis in the European bison hybrids with cattle, including 3 males of the F_1 generation and 12 males of the B_1 generation ($^3/_4$ cattle, $^1/_4$ bison). It was found that in F_1 hybrids the spermatogenesis is arrested at the stage of spermatogonia or primary spermatocytes. In three individuals of the B_1 generation spermatogenesis was arrested at the stage of spermatogonia or primary spermatocytes, in five — at the stage of secondary spermatocytes, and in other three individuals the presence of scarce spermatozoa was observed. In cases when spermatogenesis is not completed the reproductive cells undergo degeneration progressively with the increase of the animal age. Often there were observed degeneration and overgrowth of the interstitial cells with connective tissue, this fact being certainly related to the lack of stimulatory activity of hormones. Also the obliteration of capillary blood vessels was found. The relationships between the origin of hybrids and the seminiferous tubule size, as well as between the tubule size and the degree of advancement of spermatogenesis, were ascertained. In the individuals with spermatogenesis progressing until the stage of spermatozoa the mean tubule area reached ca 30,000 μ^2, whereas in hybrids with spermatogenesis arrested in early stages the tubule area amounted to barely 20,000 μ^2. The obtained results were compared with other studies concerning spermatogenesis in the bison and cattle hybrids. Various hypotheses explaining sterility of male hybrids were discussed.

I. INTRODUCTION

The problem of inter-genera and inter-species hybrids of large mammals is interesting both for stock-breeders attempting to obtain new phenotypic features and for zoologists investigating inter-species fertility, isolatory barriers between species, or generally some aspects of species and speciation. The main obstacle in the fixation of favourable features of hybrids depends on a limited fertility of the F_1 hybrids. According to Haldane's rule (1922) sterility affects always individuals of the heterogametic sex, that is males in mammals. On the other

hand, individuals belonging to the homogametic sex may be fertile in some hybrids. Dobzhansky (1964) distinguished the gene and chromosomal sterility of hybrids. In the first case sterility is caused by differences between parental genomes arising from gene mutations, in the second one — by chromosomal aberrations. Among *Bovidae* hybrids the most often encountered is the sterility of males, although some interspecies hybrids are fertile in both sexes (Gray, 1954).

The crosses between the genera of *Bison* and *Bos* were carried out in two combinations. In the Canada Agriculture Experimental Farm (Manyberries, Alberta) the bison was crossed with cattle. Males of the F_1 generation and of the first backcrosses were sterile. Normal meiotic conjugation was observed only in hybrids with 14% of bison blood. Spermatogenetic activity in seminiferous tubules was noted already in hybrids with 22% of bison blood (Peters, 1964).

Crosses of the European bison and domestic cattle were obtained in Europe already in the beginning of XIX century (Müller, 1852; Ackerman, 1898; Karcov, 1903). According to majority of authors all male hybrids of the European bison and domestic cattle are sterile in the F_1 generation (Ivanov, 1913; Zablockij, 1939; Krasińska, 1967 b, 1971). Only in the monogroph of Karcov (1903) a reference is found that Walicki obtained a fertile male in the F_1 generation after crossing the European bison and cattle of the race »szwyc« in the years 1847—1857. This reference, however, raises doubts. The main centre of breeding of the European bison and cattle hybrids before the second world war was Askania Nova, and now is Białowieża. In these centres all the males of F_1 generation were sterile. This fact should be emphasized since recently a faulty information appeared claiming that hybrids between *Bos taurus* and *Bison bonasus* are fertile (Basrur & Moon, 1967; Basrur, 1969). It is likely that the first false reference on this subject appeared in the paper by Melander (1959): »crosses between cattle and wisent have repeatedly taken place in the zoological gardens of Europe with good fertility in the F_1 hybrids...« It may be suspected that Melander had in mind crosses between *Bison bison* and *Bison bonasus*, which are common in European zoological gardens and the obtained hybrids are always fertile. On the other hand, the only zoological garden in which hybrids of the European bison and cattle were obtained is that of Płock (Poland) (Taworski & Woliński, 1960). A broad discussion of hybrids of the European bison and domestic cattle may be found in the paper by Krasińska (1967a).

Microscopic examinations of spermatogenetic activity of seminiferous tubules in the hybrids of the bison and cattle were carried out by Peters (1964) and Basrur & Gilman (in press). In the present study

there are presented results of microscopic investigations of the testes in the hybrids of *Bison bonasus* × *Bos taurus dom.* of the F_1 and B_1 generations (the latter as backcrosses in the direction of cattle).

II. MATERIAL AND METHOD

The investigations were carried out on the hybrids of the European bison with the cattle belonging to the race of lowland black and white (*bw*), or Polish red (*pr*), and in one case to the Jersey race. The hybrids were obtained in the years 1960—69 in the experimental reservation of the Mammals Research Institute PAS at Białowieża. The testes derived from 3 males of the F_1 generation in the age of 1.5 — 8 years and from 12 males of backcrosses B_1 ($^3/_4$ cattle, $^1/_4$ European bison) in the age of 0.5 — 4.5 years. The following lines were distinguished: I Line — hybrids of the F_1 generation deriving from the crosses of the European bison and domestic cow;

Table 1

List of the males hybrids of European bison × cattle studied.

Generation	Line	Name	Father	Mother	Age, in yrs.
F_1	I	Fakir	*w*	*bw*	1.5
	I	Farad	*w*	*bw*	6.5
	II	Filip	*pr*	*w*	8.0
B_1	Ia	Felon	*bw*	F_1 I	0.5
		Fenix	*bw*	F_1 I	1.5
		Feld	*bw*	F_1 I	1.5
		Fellach	*bw*	F_1 I	2.5
		Fen	*bw*	F_1 I	3.5
		Fey	*bw*	F_1 I	4.0
		Fetysz	*bw*	F_1 I	4.5
	IIa	Fest	*bw*	F_1 II	0.5
		Festyn	*bw*	F_1 II	2.5
		Feg	*bw*	F_1 II	3.5
		Feb	*bw*	F_1 II	3.5
		Fez	*bw*	F_1 II	4.5

Abbreviations: *w* — European bison; *bw* — black and white lowland breed; *pr* — Polish red breed; F_1 — hybrids $^1/_2$ European bison $^1/_2$ cattle; B_1 — hybrids $^1/_4$ European bison $^3/_4$ cattle.

II Line — animals from the inverse combination, domestic bull and bison cow; Ia Line — B_1 generation (backcross — $^3/_4$ cattle) deriving from F_1 mothers of line I; IIa Line — B_1 hybrids deriving from F_1 mothers of line II (Table 1).

The testes of two males (Fakir, Fenix) from the two generations were obtained by their castration at the age of 18 and 20 months, and of the remaining specimens after slaughtering, when they were immediately excised and fixed. For a comparison the testes of a 10 years old European bison from the free-living stock were used.

Small fragments of the testes (ca 1 cm^3) were fixed in Bouin's fluid, embedded in paraffin and sectioned to obtain slices 10—20 μ thick. The slides were stained with haematoxylin and eosin.

One hundred tubules were evaluated under microscope and the stage of spermatogenesis arresting was noted. In each individual 10 tubules representing mean spermatogenetic activity were selected and the total number of cells in all spermatogenetic stages was estimated by counting. On the basis of two measurements of the diameter of 10 tubules the average radius and then the area of the tubule were computed. The number of cells in particular stages of spermatogenesis was expressed per 1000 μ^2 in order to eliminate differences arising from variable dimensions of tubules.

III. MICROSCOPIC OBSERVATIONS OF THE TESTES

1. The European Bison

The testes of a 10 years old European bison (Fig. 2) [1]), in which normal picture of the full spermatogenesis cycle was observed, constitute the control material.

The seminiferous tubules of the European bison are large, their basement membranes adjoin on a large area, interstitial cells are scarce and fill only small spaces between tubules. The nuclei of Sertoli cells adhere to the basement membrane of tubules and their processes reach the centre of the tubule lumen. Spermatogonia form a regular layer just behind Sertoli cells, and sometimes they adjoin basement membrane. A large percentage of spermatogonia in the stage of proliferation was observed. Primary spermatocytes with clearly outlined nuclei are located in the next layer. Their number is relatively highest in comparison with other stages of spermatogenesis. Secondary spermatocytes are visible in the tubule lumen. They may be distinguished due to a smaller size of cell nuclei and they are accompanied by spermatides and spermatozoa. Spermatides are always more numerous than spermatozoa but they are jointly bounded by the processes of Sertoli cells.

2. F_1 Hybrids

The mean areas of transverse sections of seminiferous tubules in these hybrids show a considerable variability. The smallest tubules, well below those observed in the European bison, were found in Filip, intermediate — in Fakir, and the largest (but slightly smaller than in the European bison) — in Farad. The interstitial tissue was either well developed (Fakir), or degenerated and overgrown with connective tissue (Farad and Filip). The basement membrane of the tubules was markedly thicker in comparison with the European bison (the thickest was in Filip and Farad), and consisted of a few layers. The nuclei of Sertoli cells were

[1]) Figs 2—17 see Plates IV—V.

located in most cases regularly at the basement membrane, but occasionally were scattered in the whole tubule lumen (Filip). Spermatogenesis was reduced and among the germinal cells scarce spermatogonia were found (Farad, Filip; Figs 4—6). Sometimes not numerous primary spermatocytes, usually degenerated, were encountered (Fakir) (Fig. 3).

3. B_1 Hybrids (Backcrosses)

Whereas the testes of F_1 hybrids represented almost identical picture, the B_1 generation showed more diversity. In the tubules of a few individuals the germinal cells were completely lacking and only Sertoli cells were observed, but in other animals even spermatozoa occurred. Also there were found differences in the tubule diameter and in the picture of interstitial cells.

The dimensions of seminiferous tubules in B_1 hybrids showed a marked variability. In majority of individuals they were smaller than those in the European bison. The smallest ones were found in Fest, intermediate in Felon, Fenix, Fetysz and Fey, the largest (almost approaching those in the wisent) in Fez, Feb, Feld and Fen. In 5 individuals the interstitial cells were strongly degenerated and overgrown with connective tissue (Fenix, Feb, Feld, Fez, Fen), whereas in all the remaining B_1 hybrids they were normal, and this coincided with the observations on sexual behaviour in these animals. Only in Felon the interstitial cells did not fill the whole spaces between the tubules. The capillaries walls were often thickened, either slightly (Fetysz, Fey, Fest and Fez), or very strongly (Feb, Fen, Fellach, Feld; Fig. 15). The basement membrane of seminiferous tubules was thin in all individuals, but the thinnest in Fetysz, Fen and Fey (Fig. 8). The nuclei of Sertoli cells were in most cases arranged regularly at the basement membrane. In some cases the number of Sertoli cells was very high, their processes occupying the whole lumen of tubules (Felon); in other they were lying desquamated in the tubule lumen (Fenix, Fetysz, Fey; Fig. 8). Depending on the degree of spermatogenesis advancement in B_1 hybrids 4 groups of animals were distinguished: 1 — with no germinal cells (Fenix; Fig. 7); 2 — with degenerated spermatogonia only (Fetysz, Felon), or with additionally some degenerated primary spermatocytes (Fey; Fig. 9); 3 — containing both spermatogonia and primary spermatocytes, and some individuals also with considerably degenerated and not numerous secondary spermatocytes (Feg; Fig. 10, Festyn; Fig. 12, Feb, Feld; Fig. 14, Fest; Fig. 11); 4 — showing all the stages of spermatogenesis including spermatozoa (Fez, Fen; Fig. 17, Fellach; Fig. 16). In Fen several characteristic waves of spermatogenesis were even observed. Some individuals showed the pre-

sence of empty tubules containing only Sertoli cells, apart from other normal tubules with germinal epithelium (Festyn; Fig. 12, Fest; Fig. 11). Also spermiophages were encountered in the tubule lumen, either sporadically (Fest), or commonly (Feb, Fellach, Feld; Fig. 13).

4. Epidydymis

The epidydymis of the investigated hybrids was also examined, particularly in the individuals having spermatozoa in seminiferous tubules. None of the hybrids showed spermatozoa in the epidydymis and only in a few cases single spermiophages were found.

IV. SIZE OF SEMINIFEROUS TUBULES AND COURSE OF SPERMATOGENESIS

The determinations of mean areas of seminiferous tubules (Table 2) show a considerable variability. In three F_1 hybrids mean tubule areas vary in the range of 10,427.0 — 21,670.6 μ^2. A relationship between the origin of hybrids and the area of seminiferous tubules has been ascertained (Tables 1 & 2). In a F_1 hybrid deriving from the cross of domestic cattle with the European bison cow (II line) the mean area of tubules was considerably smaller than in I line hybrids. Among the individuals of I line the younger one (1.5 years old Fakir) had much smaller tubules than the older one (6.5 years old Farad). In all three cases F_1 hybrids showed a considerably reduced tubule area in comparison with the European bison (32,668.9 μ^2).

In the B_1 generation the smallest tubule area was noted in the youngest, 0.5 years old hybrids. In the Ia line it amounted to 13,033.4 μ^2, and in IIa line to 9,448.6 μ^2 (Table 2). Similarly to the F_1 generation a larger tubule area was found in the hybrids of Ia than IIa line. This regularity occurred also in older animals (Fig. 1A and Table 2). Fez (IIa line) was the only exception, because in this case a larger area of the tubule section was found than in the hybrid of Ia line (Fetysz) of the same age. This difference may arise from the fact that in Fez the tubules were significantly damaged.

It appears that there exists a relationship between the tubule size and the degree of spermatogenesis advancement (Fig. 1). It should be emphasized that in two hybrids, in which spermatozoa were found (Fen and Fellach), the size of tubules was of the same order of magnitude as in the European bison. In the third, 1.5 years old hybrid (Feld), a similar tubule area was calculated. This animal had only secondary spermatocytes, but the number of cells in particular stages of spermatogenesis per 1000 μ^2 of tubule area was similar to that found in Fen and Fellach

Table 2

The mean area of seminiferous tubules (in μ^2) and the degree of advancement of spermatogenesis.

Name	Age, years	Mean area of seminiferous tubules, in μ^2				a	b				
		Generation F^1		Generation B^1							
		I line	II line	Ia line	IIa line	Sertoli celles	Spermato-gonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa
Felon	0.5			13,033.4		36	59	5	—	—	—
Fest	0.5				9,448.6	18	17	55	10	—	—
Fakir	1.5	17,753.8				8	76	16	—	—	—
Fenix	1.5			19,173.4		+	—	—	—	—	—
Feld	1.5			34,651.2		+	1	46	53	—	—
Fellach	2.5			30,330.4		34	18	16	15	5	12
Festyn	2.5				21,075.1	+	81	18	1	—	—
Fen	3.5			36,730.1		+	1	6	38	9	46
Feb	3.5				27,793.0	+	19	72	9	—	—
Feg	3.5				13,738.9	10	70	18	2	—	—
Fey	4.0			19,420.6		67	30	3	—	—	—
Fetysz	4.5			18,646.3		83	17	—	—	—	—
Fez	4.5				35,240.2	+	+	59	20	3	18
Farad	6.5	21,670.6				66	34	—	—	—	—
Filip	8.0		10,427.0			59	41	—	—	—	—
Rison	10.0			32,668.9		+	+	+	8	60	32

—) Given stage is lacking; +) All the tubules show a given stage; a) Per cent of seminiferous tubules without reproductive cells but with Sertoli cells; b) Per cent of seminiferous tubules in which spermatogenesis was arrested at a given stage.

(Table 3). It is likely that further stages of spermatogenesis would be later developed in Feld.

The relationship between the tubule size and development of spermatogenetic stages (cf. Fig. 1) is well illustrated by the example of two hybrids 2.5 years old: Fellach (Ia line), which showed spermatozoa, and Festyn (IIa line) which had only scarce secondary spermatocytes. The mean tubule area in these hybrids amounted to 30,330.4 μ^2 (Fellach) and 21,075.1 μ^2 (Festyn). Moreover, the two oldest hybrids (4 years old Fey and 4.5 years old Fetysz), which had only spermatogonia or primary spermatocytes, showed the mean tubule size equal to 19,420.6 μ^2 and

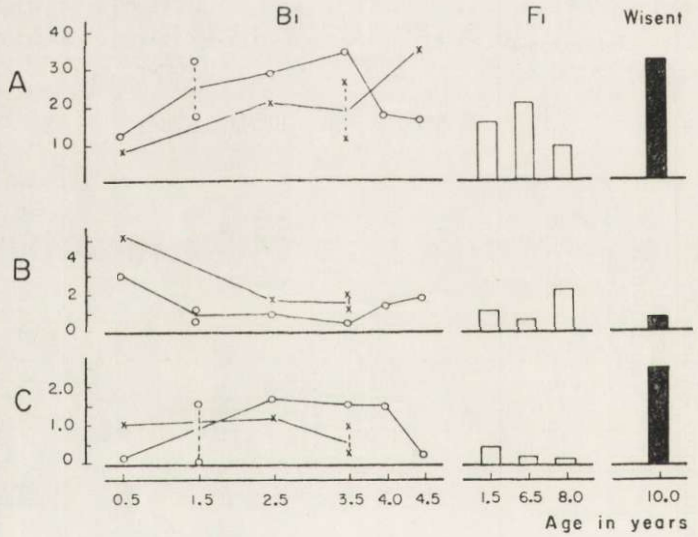

Fig. 1. The relationship between the area of seminiferous tubules and spermatogenetic activity of B_1 and F_1 hybrids and wisent. A. Mean seminiferous tubule area (in $\mu^2 \times 1000$). B. Number of Sertoli cells per 1000 μ^2 of the seminiferous tubule area. C. Total number of cells in all stages of spermatogenesis per 1000 μ^2 of the seminiferous tubule area. o — Ia line; x — IIa line.

18,646.3 μ^2, respectively. These values are of the same order of magnitude as in 1.5 years old Fenix which had entirely empty tubules. From this last fact it can be concluded that the tubules reach the maximum size in the Ia line individuals in the age of 1.5 years. Then, in these hybrids in which due to disturbances in the spermatogenesis processes the germinal cells are not formed, the seminiferous tubules are reduced in size. Hence in a case when germinal cells do not fill the tubule lumen, tubule walls undergo contraction. A secondary effect of this phenomenon depends on the occurrence of a very high number of Sertoli cells per 1000 μ^2 of

tubule area in these B_1 hybrids in which tubules show the smallest area (*e.g.* Fest: 5.048, Felon: 3.391, Feg: 2.089, whereas in he remaining B_1 hybrids approximately 1.000 Sertoli cells fell for the area of 1000 μ^2 — Table 3). For this reason the differences in the mean number of Sertoli cells falling for an individual tubule in the same animals are considerably smaller (Table 3).

The results of calculations of the cell number in particular stages of spermatogenesis per 1000 μ^2 of tubule area are accumulated in Table 3. Similarly to the cases of Sertoli cells also large differences exist in the number of germinal cells in particular stages of development.

Table 3

Cell number in particular stages of spermatogenesis per 1000 μ^2 of the tubule area.

Generation	Name	Mean no. of Sertoli cells falling one tubule	Mean number of cells per 1000μ^2 of the tubule area						
			Sertoli cells	Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa	Total no. of germinal cells
F_1	Filip	25.4	2.436	0.115	—	—	—	—	—
	Farad	21.2	0.690	0.038	—	—	—	—	—
	Fakir	19.9	1.121	0.310	0.028	—	—	—	0.338
	Fenix	27.5	1.434	—	—	—	—	—	—
	Fetysz	37.1	1.990	0.032	—	—	—	—	—
	Fey	29.8	1.534	0.129	0.031	—	—	—	0.160
	Felon	44.2	3.391	0.314	0.008	—	—	—	0.322
B_1	Feb	37.2	1.338	0.640	0.302	0.003	—	—	0.983
	Feg	28.7	2.089	0.204	0.022	0.007	—	—	0.237
	Festyn	29.5	1.400	1.177	0.024	0.005	—	—	1.206
	Feld	28.5	0.822	1.079	0.470	0.052	—	—	1.601
	Fest	47.7	5.048	0.518	0.423	0.095	—	—	1.036
	Fez *)	+	+	+	+	+	+	+	
	Fen	24.6	0.670	0.781	0.411	0.128	0.117	0.204	1.641
	Fellach	21.7	0.715	0.588	0.903	0.353	0.018	0.013	1.875
	Wisent	18.0	0.551	0.643	1.080	0.768	0.695	0.413	2.499

*) Damage to tubules does not permit of an accurate calculation of particular stages of spermatogenesis.

A normal picture of spermatogenesis is shown by tubules of the European bison (Table 3), in which the number of Sertoli cells and of particular spermatogenetic stages per 1000 μ^2 is similar, with the most abundant primary spermatocytes — 1.080, while the numbers of the remaining stages range from 0.413 (spermatozoa) to 0.768 (secondary spermatocytes). The number of Sertoli cells does not exceed that of reproductive cells.

The picture of spermatogenesis most similar to that in the European bison was found in tubules of two B_1 hybrids (Fellach and Fen). In the

former primary spermatocytes were most abundant (0.903), in the latter — spermatogonia (0.781). In both hybrids spermatozoa were present but their number per 1000 μ^2 of the tubule area was considerably smaller than in the European bison and amounted to 0.013 (Fellach) and 0.204 (Fen).

A somewhat similar picture was shown by the tubules of a young (1.5 years old) B_1 hybrid — Feld. In this case the spermatogenesis progressed until the formation of secondary spermatocytes. The mean tubule areas in these three discussed hybrids were of the same order of magnitude as in the European bison, *i.e.* over 30,000 μ^2 (Table 2).

In the remaining hybrids there exist marked differences in the cell number in particular stages of spermatogenesis. In a group of 5 hybrids, in which spermatogenesis reached only the stage of secondary spermatocytes, the highest number of cells in this stage was noted in the youngest individuals: 0.5 years old (Fest) — 0.095, and 1.5 years old (Feld) — 0.052. The other three hybrids in the age over 2.5 years showed the number of secondary spermatocytes in the range from 0.003 to 0.007 cells per 1000 μ^2 (Table 3). In these hybrids a marked degree of spermatocytes degeneration was observed.

The lowest number of Sertoli cells was noted in the European bison, both in respect of the mean number per 1 tubule and of the mean number per 1000 μ^2 of tubule area (Table 3). In a group of three hybrids containing at most primary spermatocytes in the tubules (Fetysz, Fey, Felon) the numbers of Sertoli cells showed the smallest differences. The number of spermatogonia and spermatocytes corresponded to those in the previous group of hybrids. In Fenix, which had only Sertoli cells, their number was the same as in Fey (Table 3), and the tubule size also corresponded to each other (Table 2).

V. DISCUSSION

In respect of fertility of hybrids between the European bison and domestic cattle there exist only scarce reports, and moreover they are not based on the analysis of semen or histological studies of the testes. Only Ivanov (1913) examined the semen of a F_1 hybrid (European bison × cow of he grey Ukrainian breed) obtained at Askania Nova and found that both the semen and tubules of the testis and of epidydymis were lacking spermatozoa.

The description of the structure of the European bison testes given by us in order of comparison with that of hybrids remains in a complete agreement with an earlier report (Kulagin, 1932).

The first generation of hybrids of the domestic cattle with the European bison, or with the bison, was entirely sterile (Ivanoff, 1911;

Ivanov, 1913; Boyd, 1914; Deakin *et al.*, 1935; Logan & Sylvestre, 1950; Zablockij, 1939, 1956; Peters, 1964; Krasińska, 1967b). On the other hand, the reports on the fertility of male hybrids from the first generation of backcrosses of both European bison and bison with cattle (3/4 European bison, 3/4 American bison or 3/4 domestic cattle) obtained in Askania Nova (Ivanoff, 1911; Ivanov, 1913; Zablockij, 1939, 1956) were not confirmed in the Białowieża experiment (Krasińska, 1967b, 1971).

The data concerning testes structure and the course of spermatogenesis in the hybrids of *Bison bonasus* × *Bos taurus* may be only compared with similar results obtained for the hybrids of *Bison bison* × *Bos taurus*. It was established that in the hybrids showing 22 and 14% of bison blood the spermatogenesis progresses normally. However, no spermatozoa were found in the epidydymis, this fact being probably related to the presence of spermiophages in the tubule lumen (Peters, 1964; Basrur, 1969). A similar spermatogenesis picture was observed in 3 Białowieża hybrids with 25% of the European bison blood, and they also contained spermiophages.

In the hybrids with 31% of bison blood the lack of spermatogenesis was ascertained (Basrur, 1969). An analogous result was obtained with the seminiferous tubules of backcrosses of the European bison and cattle. It was found that the lack of germinal cells is accompanied by a considerable reduction of the tubule section area. Moreover, some pathological changes were observed: thickening of the basement membrane of the seminiferous tubule, overgrowth of interstitial cells with connective tissue, obliteration of the blood vessel lumen. The latter feature observed in the testes of the hybrids of the European bison and cattle is almost certainly associated with hybrid sterility because it affects directly the nutritive conditions of germinal cells.

Hitherto various attempts were made in order to explain the phenomenon of sterility of hybrid males. Deakin *et al.* (1935) suggested that the disturbances of spermatogenesis are caused by a higher temperature in the scrotum of hybrids. This was denied by the investigations of Peters & Newbound (1957), who did not demonstrate statistically significant differences in the intratesticular temperature of bisons, bulls and cattalo, the latter even showing the lowest temperature. On the other hand, Peters (1964) demonstrated that there exists differentiation in the rate of postnatal development of the testes in bisons and bulls, and that in hybrids it attains an intermediate value.

Some hope for the explanation of the hybrid sterility was also associated with differences in karyotypes (mainly of Y chromosomes in parental species) (Basrur & Moon, 1967). From the comparison of

the *Y* chromosome morphology in certain *Bovinae* species containing $2N = 60$ (*Bison bison, Bison bonasus, Bos taurus* and *Bos indicus)* it arises that structural differences between *Y* chromosomes of these species are not sufficient to explain the sterility of male hybrids (F e d y k & S y s a, 1971).

In the crosses between *Bison bonasus* and *Bos taurus* the disturbances of spermatogenesis concern mainly the reductional division. In most hybrids lacking complete spermatogenetic cycle no spermatocytes were formed. On the other hand, all hybrids (except Fenix) contained spermatogonia (Table 2). These facts might suggest that sterility is caused by abnormal conjugation due to the lack of a sufficient homology of chromosomes in parental forms, or by disturbances in the divisional spindle formation in spermatocytes. These suppositions are in agreement with the observations of P e t e r s (1964) who found in the hybrids with 22% of bison blood the presence of 60 univalents in 5 cases out of 72 studied meiotic metaphases.

It must be, however, remembered that even the ascertainment of the total lack of homology between parental genomes is not sufficient to explain the sterility limited to hybrid males only. The normal fertility of F_1 hybrid females indicates the occurrence of a sex-linked trait.

It appears that more important may be a difference in time that is essential for *DNA* replication in the *Y* chromosome. In *Bos taurus* the replication of chromosome *Y* occurs at least 2 hours later than of the remaining chromosomes. However, in order to confirm the hypothesis on the importance of differences in the period of *DNA* synthesis in the *Y* chromosome for the sterility of males further comparative autoradiographic studies are required.

Moreover, some non-chromosomal factors may influence the course of spermatogenesis. As shown in Fig. 1 the maximum spermatogenetic activity was found in hybrids in the age af 2.5 — 3.5 years (the highest number of germinal cells in all spermatogenetic stages: Fig. 1C), hence in the individuals sexually mature (K r a s i ń s k a, 1971). On the other hand, in older animals a reduction in the number of germinal cells was observed. It may be supposed that in older hybrids some disturbances in the gonadotropic regulation of the hypophysis and hypothalamus occur and in effect along with the decrease of spermatogenetic activity a reduction of interstitial cells and overgrowth with connective tissue are observed. The latter phenomenon might indicate the lack of hormonal stimulation.

On the other hand, the degeneration of interstitial cells was observed also in the hybrids with developed spermatozoa (Fen and Fez). Moreover, the cryptorchic testes of the pig and horse showed not only the lack of

spermatogenesis and decrease of the seminal tubule area but also degeneration of the interstitial tissue and overgrowth of the connective tissue (Zioło & Rubaj, 1970).

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Mammals Research Institute,
Polish Academy of Sciences,
Białowieża, Poland.

Stanisław FEDYK i Małgorzata KRASIŃSKA

BADANIA NAD SPERMATOGENEZĄ HYBRYDÓW ŻUBRA Z BYDŁEM DOMOWYM

Streszczenie

W pracy przedstawiono wyniki badań nad spermatogenezą hybrydów żubra z bydłem domowym uzyskanych w Zakładzie Badania Ssaków PAN w Białowieży. Do badań użyto jądra trzech samców pokolenia F_1 w wieku 1,5 — 8 lat i 12-u pokolenia B_1 ($^3/_4$ bydła, $^1/_4$ żubra) w wieku 0,5 — 4,5 lat (Tabela 1). Materiał kontrolny stanowiły jądra 10 letniego żubra.

Obliczono średnią powierzchnię kanalików nasiennych i liczbę komórek w poszczególnych stadiach przypadającą na 1000 μ^2 powierzchni kanalików (Tabele 2, 3).

U hybrydów F_1 spermatogeneza zatrzymuje się na poziomie spermatogoniów lub spermatocytów I rzędu. W pokoleniu B_1, podobnie jak w F_1, spermatogeneza zatrzymuje się u trzech osobników na stadium spermatogoniów lub spermatocytów I rzędu, u 5 sztuk natomiast na stadium spermatocytów II rzędu i u trzech osobników

obserwowano nieliczne plemniki (Tabela 3). Jednak u osobników u których występowały w kanalikach nasiennych plemniki przewody wyprowadzające najądrza były puste. Jeżeli spermatogeneza nie dochodzi do końca to wraz z wiekiem zwierząt komórki rozrodcze ulegają degeneracji.

Stwierdzono związek między pochodzeniem mieszańców a wielkością kanalików nasiennych (Tabele 1, 3). Średnia powierzchnia kanalików jest znacznie większa u osobników pokolenia F_1, których ojcem był żubr, oraz u tych hybrydów B_1, których matki miały ojca żubra (Tabela 3).

Istnieje ponadto zależność między stopniem zaawansowania spermatogenezy a rozmiarami kanalików. U osobników u których spermatogeneza przebiega do końca powierzchnia kanalików sięga około 30,000 μ^2, natomiast u tych u których zatrzymuje się we wczesnych stadiach powierzchnia kanalików nasiennych nie przekracza 20,000 μ^2 (Tabela 2).

Pełny obraz spermatogenezy stwierdzono tylko u trzech hybrydów pokolenia B_1. U pozostałych istnieje duże zróżnicowanie ilości komórek poszczególnych stadiów spermatogenezy (Tabela 3).

Uzyskane wyniki porównano z danymi na temat spermatogenezy hybrydów bizona i bydła. Przedyskutowano również hipotezy dotyczące sterylności samców — hybrydów.

EXPLANATION OF PLATES

Plate IV

Fig. 2. Cross section of a normal seminiferous tubule of the wisent showing the full cycle of spermatogenesis.

Fig. 3. Cross section of a testis from F_1 hybrid (Fakir). Total lack of spermatogenesis is characteristic of the seminiferous tubules from this animal.

Figs 4, 5. Cross sections of the testis from F_1 hybrid (Filip) showing the Sertoli cells only.

Fig. 6. Section of a small blood vessels from testis of F_1 hybrid (Filip).

Fig. 7. Cross section of a testis from B_1 hybrid (Fenix) showing absence of spermatogenesis and the amorphous deposit in the light of seminiferous tubules.

Figs 8, 9. Cross sections of a testis from B_1 hybrid (Fey). Lack of spermatogenesis (Fig. 8) and presence of primary spermatocytes (Fig. 9) are noted.

Plate V

Fig. 10. Cross and oblique sections of seminiferous tubules from the testis of B_1 hybrid (Feg).

Fig. 11. Cross and oblique sections of seminiferous tubules from the testis of B_1 hybrid (Fest), showing primary and secondary spermatocytes.

Fig. 12. Section of a testis of B_1 hybrid (Festyn).

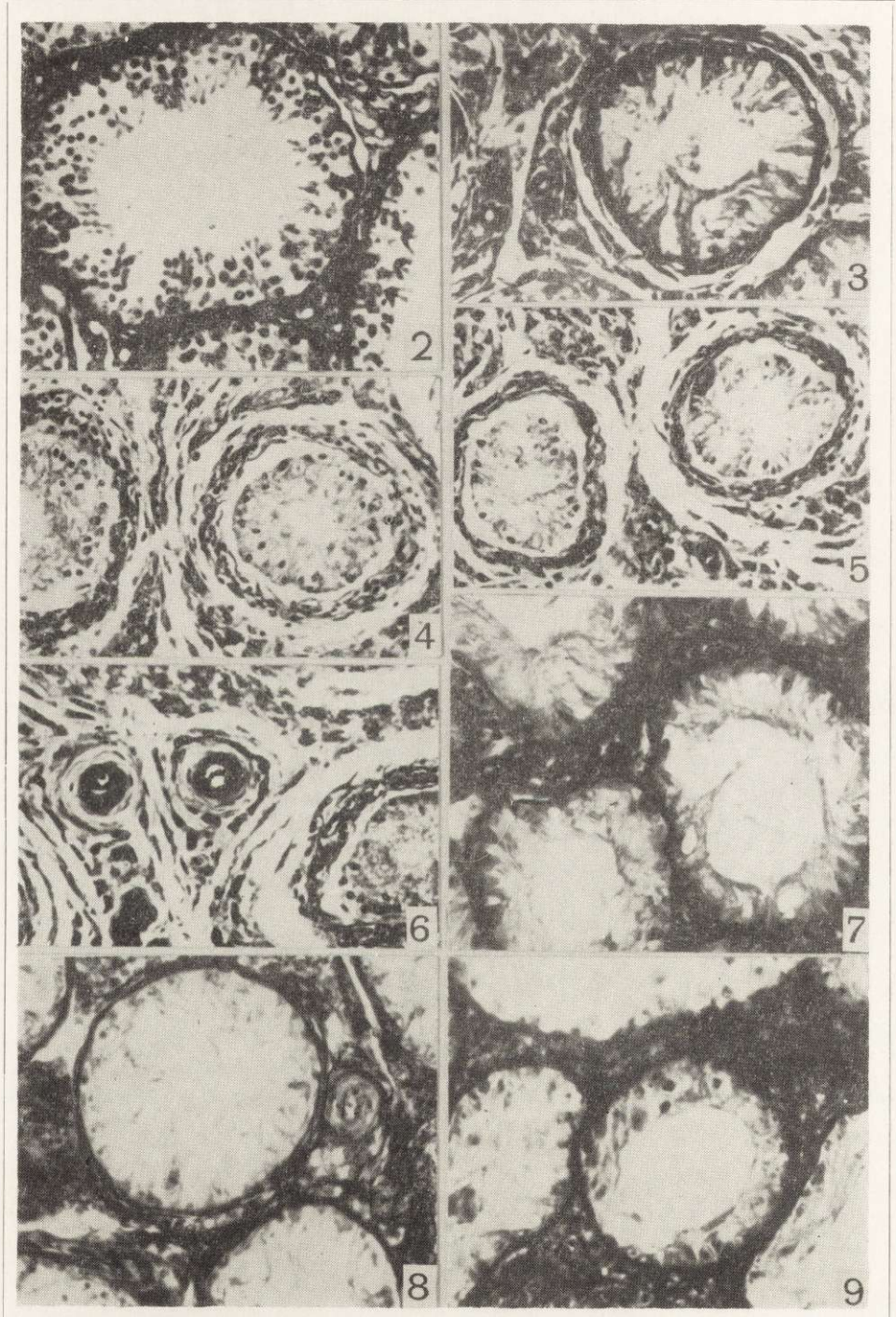
Fig. 13. Section of a testis of B_1 hybrid (Feld) showing a presence of spermiophage (arrow).

Fig. 14. Cross section of a seminiferous tubule from B_1 hybrid (Feld) with the Sertoli cells, and primary and secondary spermatocytes.

Fig. 15. Longitudinal section of blood vessel from the testis of B_1 hybrid (Feld).

Fig. 16. Cross section of a seminiferous tubule from the testis of B_1 hybrid (Fellach). All stages of meiosis and spermatogenesis were noted in this hybrid.

Fig. 17. Cross section of seminiferous tubule from the testis of B_1 hybrid (Fen), with all stages of spermatogenesis including the spermatozoa.



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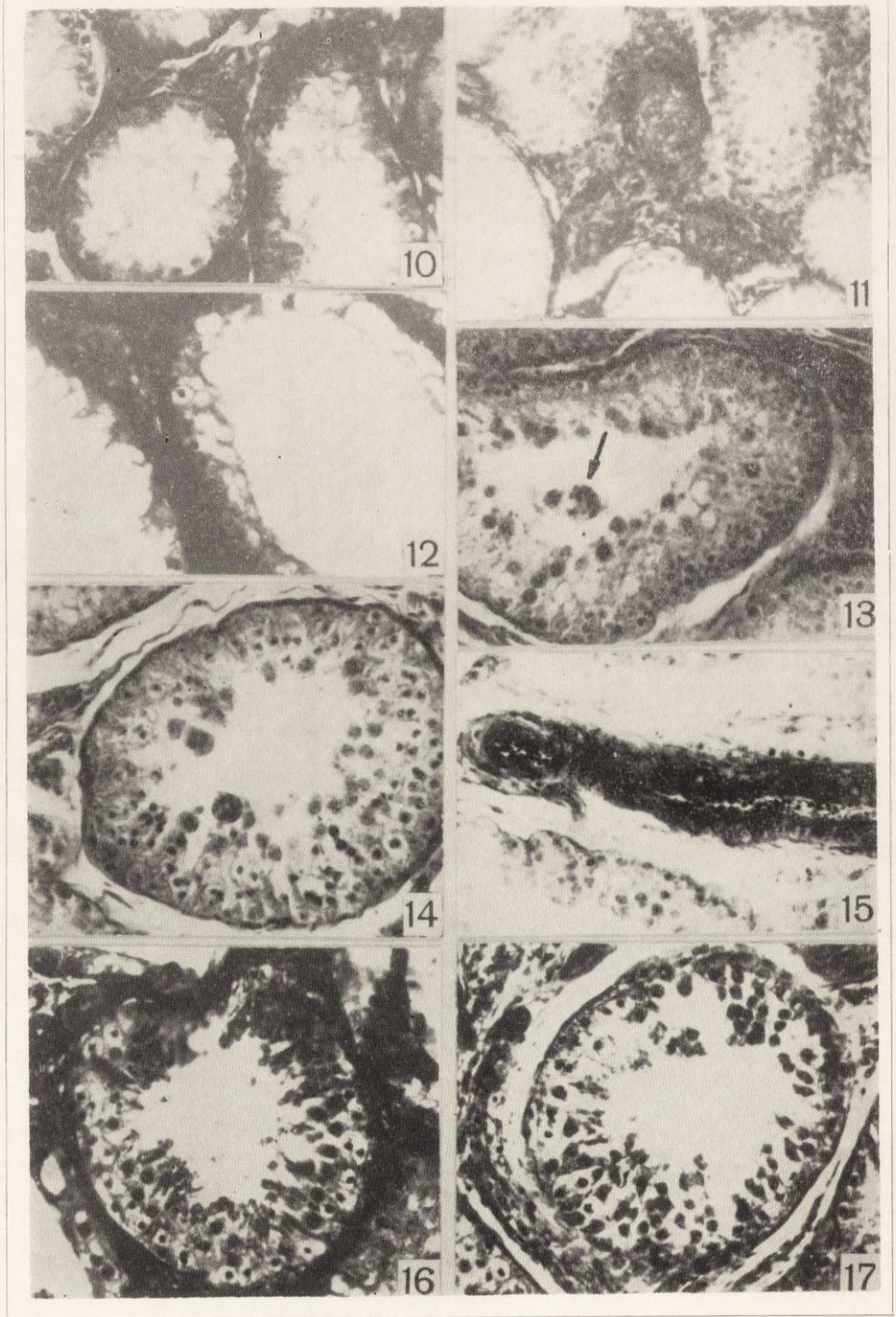

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