Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2019) 43: 433-440
© TÜBİTAK
doi:10.3906/vet-1901-95

Analysis of spermatogenesis in young male hybrids bred from domestic cattle (*Bos taurus*) and European bison (*Bison bonasus*)

Andrzej ŻYCZYŃSKI*, Marta KLOCH, Wanda OLECH
Department of Genetics and Animal Breeding, Faculty of Animal Science, Warsaw University of Life Sciences, Warsaw, Poland

Received: 30.01.2019 • **Accepted/Published Online:** 03.07.2019 • **Final Version:** 07.08.2019

Abstract: A new attempt was undertaken to check the reproductive capabilities of male hybrids bred from European bison (*Bison bonasus*) and domestic cattle (*Bos taurus*). The analysis concerned two hybrids aged 1.5–2.5 years (the latter being sampled twice at 6-month intervals), and additionally one 1.5-year-old backcross hybrid. Examination of testicular tissue revealed halting of spermatogenesis, which was hardly ever able to proceed further than meiosis I. Spermatogenesis was arrested at the stage of spermatogonia and primary spermatocytes. Spermatozoa were found only in the case of one hybrid and these were mostly malformed. To summarize, all the males studied were sterile, despite the evident sexual behavior exhibited by one individual.

Key words: *Bison bonasus*, *Bos taurus*, spermatogenesis, hybridization

1. Introduction

The family Bovidae has been widely exploited by humans since the dawn of mankind. Their uses have been diverse and include the hunting of wild animals, breeding of farm animals, beasts of burden, production of milk and meat, and as a source of such raw materials as leather, glands, and bones.

With the development of human settlements and the beginning of agriculture, most of the Bovidae species were domesticated and improved strains were bred. Careful selection led to substantial improvements in productivity and this is continued today, particularly within herds of milk-producing breeds, Friesian cattle being the most spectacular example [1]. The Murrah breed of Asiatic buffalo (*Bubalis bubalis*) is another example where selective breeding has paid off [2].

Another method of genetic improvement is the breeding of hybrids. Attempts have been undertaken in this field for cross-breeding between species, subspecies, and even genera. According to Linnaeus' definition, generally speaking only hybrids bred from species of the same genus are expected to be fertile. However, in the case of Bovidae, the females of hybrids bred from *Bos* and *Bison* genera are fertile, while the males are not. It is interesting that the same applies to hybrids bred from domestic cattle (*Bos taurus*) and the Asiatic yak (*Bos grunniens*). According to Ritz et al. [3] and Verkaar et al. [4], the genetic relationship between the Asiatic yak and American bison (*Bison bison*) is closer than between the yak and domestic cattle, so some taxonomic revision in the near future is quite possible.

The infertility of male hybrids remains an intriguing phenomenon. To begin with, the reason for this was sought on the chromosomal level. All the species of interest have diploid number 2n = 60, and chromosomes are acrocentric except for sex chromosomes. There are no differences between autosomes and X chromosomes among the species examined. In European domestic cattle (*Bos taurus*) and Asiatic yak, the Y chromosome is small and metacentric. In Asiatic zebu cattle (*Bos indicus*) and American bison (*Bison bison*), the Y chromosome is small and acrocentric, and this shape is believed to be the result of pericentric inversion of the type of Y chromosome seen in *Bos taurus*. In the case of European bison (*Bison bonasus*) the Y chromosome was first described as small submetacentric [5] and later as small acrocentric [6,7]. Accordingly, the male's bivalent sex chromosome was the obvious candidate as the cause for the malfunction of meiosis in hybrids and for preventing completion of spermatogenesis. However, male hybrids bred from European cattle and zebu cattle are fertile despite the different morphology of their Y chromosomes. On the contrary, male hybrids bred from yak and cattle (both European and zebu) are infertile, but for easier handling they have to be castrated when used as beasts of burden. The fact that yak and European cattle have identical Y chromosomes makes no difference at all. Male hybrids bred from cattle bulls and American bison

* Correspondence: andrzej_zyczynski@sggw.pl

(called cattalo) are sterile, just as are hybrids bred from cattle bulls and European bison. The use of zebu cattle or European bison males for cross-breeding with American bison (with identical acrocentric Y chromosomes) makes no difference; the male hybrids are sterile. Thus, the morphology of the Y chromosome is clearly not the reason for spermatogenesis failure in hybrids.

The aim of this study was to describe the spermatogenesis of hybrids bred from domestic cattle and European bison.

2. Materials and methods

The research was carried out on two hybrids (F1 generation) and one backcross animal (B1 generation) (Table 1). The F1 hybrids were the offspring of European bison and domestic cattle of the Holstein Friesian (HF) breed (1/2 European bison and 1/2 cattle). The B1 animal was the offspring of an F1 hybrid female and the domestic male cattle of an HF breed (1/4 European bison and 3/4 cattle). These animals were the property of the Karolew Farm, near Poznań (Poland). They were raised and fattened for production purposes. We were granted access to them during their slaughter, and in one case during a biopsy of the testes ordered by the animal's owners. No procedure requiring approval from the university's ethics commission was necessary for the purpose of this study. One hybrid (Dima) was therefore sampled twice supravitally at 2 years of age and then post mortem, 6 months later. As a reference we used testes preparations obtained from a domestic cattle bull slaughtered at approximately 1.5 years old and a European bison male at 3.5 year old culled in the Białowieża National Park. The samples were transported to a laboratory in RPMI culture medium, packed in ice, and processed according to Peters et al. [8]. Additionally, samples of testes were placed in Bouin's fixative, then dehydrated and finally immersed in paraffin. Paraffin sections of 10–20 µm were then stained with hematoxylin and eosin for microscopic examination.

The measurements of the mean area of seminiferous tubules were estimated based on the procedure described by Fedyk and Krasińska [9]. Each circumference of seminiferous tubules in the figures was outlined manually and the surface was counted with NIS-Elements AR 2.10 software, on a Nikon Eclipse 90i microscope connected to a DS5-U1 digital camera (Nikon Corporation, Tokyo, Japan). Within measured tubules surfaces, all cells of interest were counted manually.

3. Results

Preparations from the domestic cattle bull and European bison revealed spermatogenesis (Figures 1a, 1b, and 2, respectively). The preparations made from the former, the 1.5-year-old hybrid (Edward) showed only scant spermatozoa (about 20 per slide) (Figure 3a), and many of those were seriously malformed (Figure 3b). One cell with synaptonemal complex was detected but this was of very poor quality (Figure 3c). Figure 3d shows sections through this subject's seminiferous tubules. The yield of cells undergoing meiotic division was no greater in older males. On the contrary, no spermatozoa or other cells were found during meiosis on preparations made according to the procedure described by Peters et al. [8]. Figures 4a and 4b show sections through seminiferous tubules from a 2-year-old hybrid (Dima). In Figure 4c, only a single spermatocyte was found in the closest proximity to the basement membrane (light microscopic resolution). The testes of the same animal sampled 6 months later were even less active than before, as illustrated in Figures 5a and 5b. Preparations made from the testes of a 1.5-year-old (Kropek), the backcross of a hybrid female with a domestic cattle bull, showed some meiotic activity, but still not sufficient to result in fertility (Figures 6a–6c).

Table 1. List of the studied animals. Eb: European bison, Bt: cattle, F1: 1/2 European bison and 1/2 cattle, B1: 1/4 European bison and 3/4 cattle.

Generation	Name	Sire	Dam	Age in years
-	D. cattle	Bt	Bt	1.5
-	E. bison	Eb	Eb	3.5
F1	Edward	Eb	Bt	1.5
F1	Dima	Eb	Bt	2
B1	Kropek	Bt	F1	1.5

Table 2 contains the results of microscopic analyses of the seminiferous tubules of the animals examined. In hybrids derived from crossing a domestic cattle cow with a European bison bull, the mean area of tubules was greater than in the backcross. The mean area of seminiferous tubules in the hybrid Edward at the age of 1.5 years was 16762.91 µm^2. For comparison, the mean area in the hybrid named Dima at the age of 2 years was 16648.12 µm^2, and at the age of 2.5 years was 19217.85 µm^2. In this case, the mean area of seminiferous tubules increased with age. The mean area of tubules in the 1.5-year-old backcross named Kropek was 16473.97 µm^2. As for the number of cells, the greatest number was observed in the backcross animal, with the lowest observed in Edward, one of the 1.5-year-old hybrids. In terms of cell diversity (Table 3), the least diversity was observed in the hybrid named Dima. In this hybrid's first measurement at the age of 2, most of the Sertoli cells and residual spermatogonia were identified. In the second measurement (aged 2.5), only Sertoli cells were found. Among all the hybrids, the greatest cell

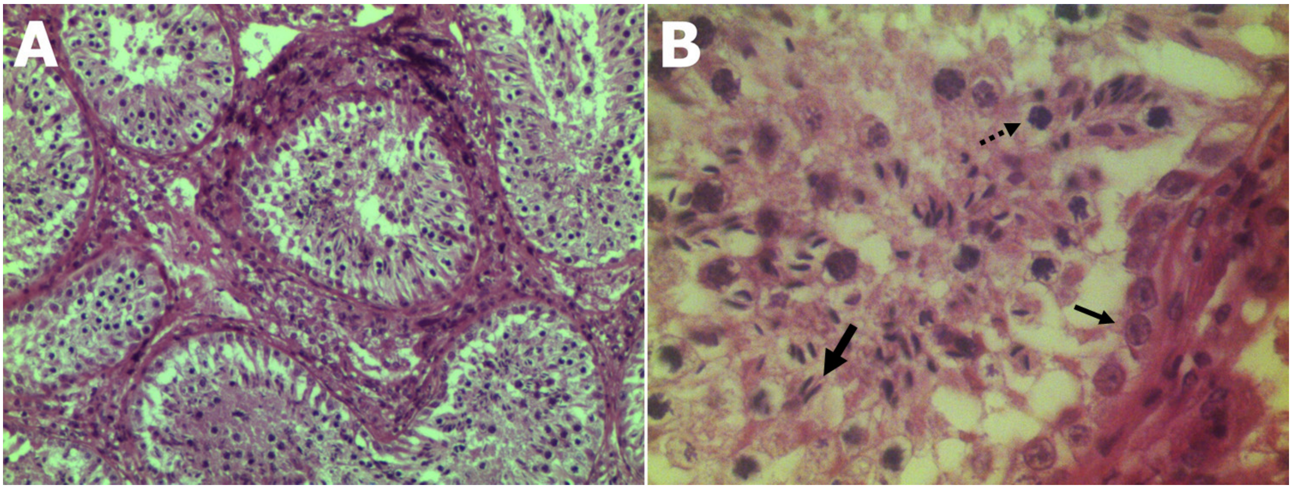

Figure 1. Photograph (A) showing the seminiferous tubules of an adult domestic cattle bull (*Bos taurus*) (H&E stain, 40×) and (B) showing a fragment of the seminiferous tubules of an adult domestic cattle bull (*Bos taurus*). Numerous spermatids (larger arrow), Sertoli cells (smaller arrow), and primary spermatocytes (dotted arrow) are visible (H&E stain, 100×).

diversity was observed in a 1.5-year-old, where 50% of the cells were Sertoli, 36% spermatogonia, and 14% primary spermatocytes. In the backcross individual, 54% of cells were Sertoli, 45% spermatogonia, and only 1% primary spermatocytes. No secondary spermatocyte, spermatids, or spermatozoa cells were detected in any of the tested animals. An exception was the 1.5-year-old hybrid, where a negligible number of cells in the spermatozoa stage were detected, but as previously mentioned, these were seriously malformed. The above-mentioned spermatozoa were found only on slides prepared according to Peters et al. [8], but not on histological preparations.

4. Discussion

Since analyses of the synaptonemal complex of hybrid males were not possible, due to lack of appropriate materials, this research focused on microscopic analysis of testes among hybrids of various ages and degrees of breeding activity.

The first trial involving a 1.5-year-old hybrid failed to provide any of the required data. In the case of this male, although some sperm were found (Figure 3a), most were malformed (Figure 3b). However, this could have been the result of a staining procedure not aimed at preservation of spermatozoa—see the method of Peters et al. [8]. Only one cell with synaptonemal complex at pachytene stage was detected, but this was of very poor quality (Figure 3c), insufficient for any conclusion to be made. The poor yield of cells of interest may have been due to the young age of the male, since domestic cattle males are normally fertile at the age of 1.5 years and can reach sexual maturity as early as 12 months, whereas European bison take much longer to mature. Krasińska and Krasiński [10] found that

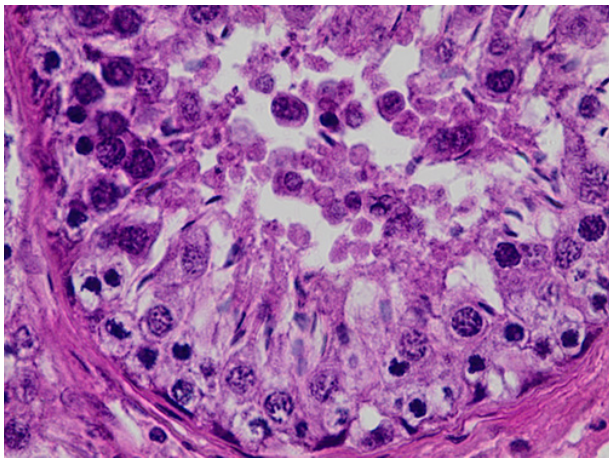

Figure 2. Photograph of a fragment of the seminiferous tubules of a European bison (*Bison bonasus*) showing meiotic activity in the nonbreeding season (H&E stain, 100×).

male European bison are rarely mature at the age of 2, with maturity usually being reached in the 3rd year and full maturity being achieved by the 4th. The age at which maturity is reached depends to some extent on body weight. It may therefore be assumed that male hybrids take longer than cattle bulls to mature. Unfortunately, expectations concerning the use of material from older males – i.e. 2 and 2.5 years old, proved to be unfounded.

The hybrid male described by Krasińska [11] was very aggressive towards other males and exhibited a strong sexual urge during estrus in cows. Similar behavior was observed in the 2-year-old hybrid (Dima) on the Karolewo Farm. This explains the necessity of castrating male hybrids

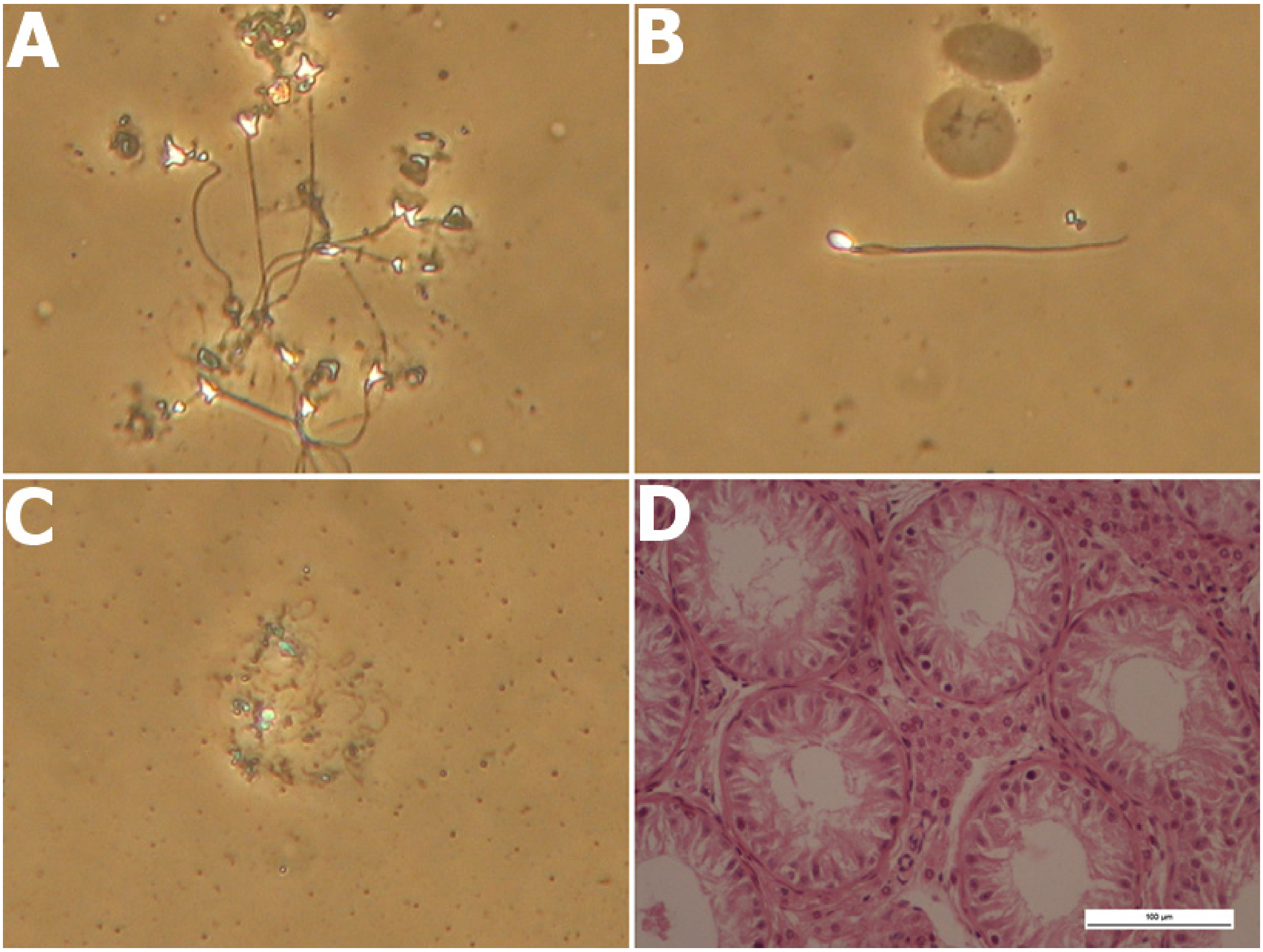

Figure 3. Photograph showing preparations from a 1.5-year-old hybrid (Edward): (A) typical spermatozoon (100×), (B) a group of malformed spermatozoa (100×), (C) a meiotic cell at the pachytene stage in which synaptonemal complex can be seen (100×), (D) seminiferous tubules (H&E stain, 40×).

bred from yak and domestic cattle, as widely practiced by Nepalese porters and shepherds. However, this aggressive sexual behavior was not reflected in the spermatogenesis in our materials (Figures 4a–4c). Single spermatogonia could be seen in very few sections through seminiferous tubules from the 2-year-old hybrid. Only one single spermatocyte close to the basement membrane was found, in addition to connective tissue separating tubules. The second sampling of the same hybrid 6 months later showed that its fertility did not increase with age (Figures 5a and 5b). Moreover, no germinal cells whatsoever were detected. Only Sertoli cells were found on the seminiferous epithelium. This confirms the results of Fedyk and Krasińska [9], which showed that spermatogenesis diminished as the hybrids studied grew older.

Analysis of the 1.5-year-old backcross, and likewise a 1.5-year-old domestic cattle specimen, showed more germinal cells than in the 2.5-year-old hybrid (Figures 6a–6c). Primary spermatocytes and cells in the prophase stage are seen, but not in any further stages. Unfortunately, no material from older backcross animals was available to determine whether spermatogenesis diminishes in the same manner as in older (2.5-year-old) hybrids. All results obtained in this study accorded with the data presented by Fedyk and Krasińska [9,12], who conducted a broad comparative histomorphological analysis of testes tissue, together with a literature survey. In all the cases studied, even where rudimentary spermatogenesis was detected (in a young 1.5-year-old hybrid), this was not enough to ensure fertility. However, those authors found completed spermatogenesis in backcross B2 and B3 generations. All stages of spermatogenesis were more frequently detected with a greater percentage of cattle genes in backcrosses. Unfortunately, Fedyk and Krasińska [9,12] were unable to check the actual amounts of spermatozoa in the backcrosses studied and likewise this study could not include an analysis

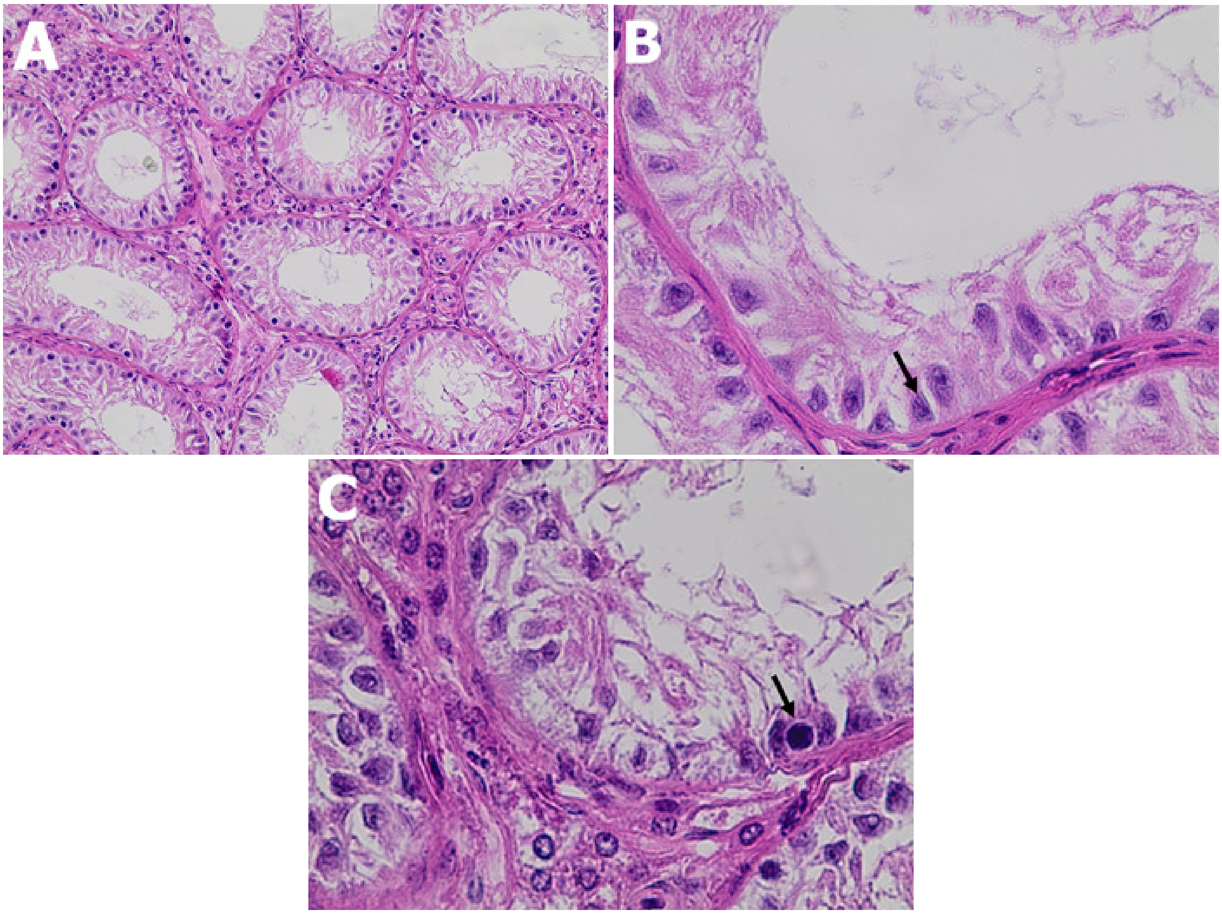

Figure 4. Photograph showing preparations from a 2-year-old hybrid (Dima): (A) seminiferous tubules (H&E stain, 40×), (B) fragment of the seminiferous tubules (H&E stain, 100×). Germinal epithelium can be seen—Sertoli cells only (large arrow). (C) Fragment of the seminiferous tubules. Also visible are a single spermatocyte (large arrow) close to the basement membrane and connective tissue separating tubules (H&E stain, 100×).

of spermatozoa in the youngest hybrid (1.5-year-old Edward).

Since the female hybrids among the studied species are fertile, the failure of gametogenesis is obviously sex-linked, although it is difficult to explain impotence at the cytogenetic level in terms of bivalent sex chromosome.

Perhaps the imperfect conjugation of homologues of differing genera or errors in their division in anaphase could have been one of the reasons for the first meiosis being interrupted.

Peters [13] found numerous univalents in meiotic studies of metaphase I in backcrosses of cattalo males. Another recently proposed cause is disruption of the hypothalamic–pituitary axis, presumably in receptor cell activity. However, those few cells that, despite shortcomings, do proliferate, face other obstacles, such as excessive growth of dense connective tissue and basement membrane (Figures 4a and 4b), potentially weakening blood vessels by restricting their lumen, which in turn may lead to reduced nutrition conditions.

Despite the great expectations placed on the Karolewo Farm, this study again confirmed the total sterility of male hybrids bred from the genera *Bos* and *Bison*.

Today, when healthy, unprocessed, and more diversified food is popular, attempts are being made to lend economic significance to the production of hybrids bred from domestic cattle and bison. Łozicki et al. [14] conducted a comparative evaluation of the meat-producing performance of hybrids bred from European bison and domestic cattle. The results clearly emphasized the nutritive value and quality of the meat from hybrids. Nevertheless, our study once again confirms that in order to produce hybrids, crossbreeding must each time be carried out between the parent species, because only one sex of a hybrid, the female, is fertile. Crossbreeding must therefore be repeated in each production cycle. A separate issue seems to be the existence of spontaneously crossbred populations. When in North America the bison was on the verge of extinction, crossbreeding of bison and cattle

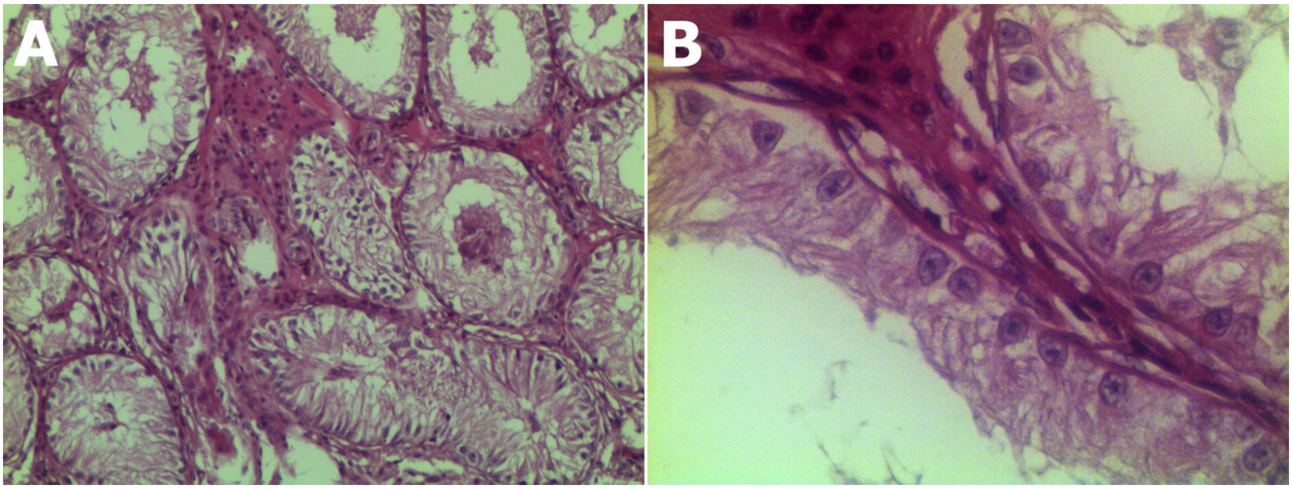

Figure 5. Photograph showing preparations from a 2.5-year-old backcross (Dima): (A) seminiferous tubules—meiotic activity is lower than 6 months earlier (H&E stain, 40×), (B) a fragment of the seminiferous tubules—the effect of cellular detritus activity. Still no meiotic activity is visible (H&E stain, 100×).

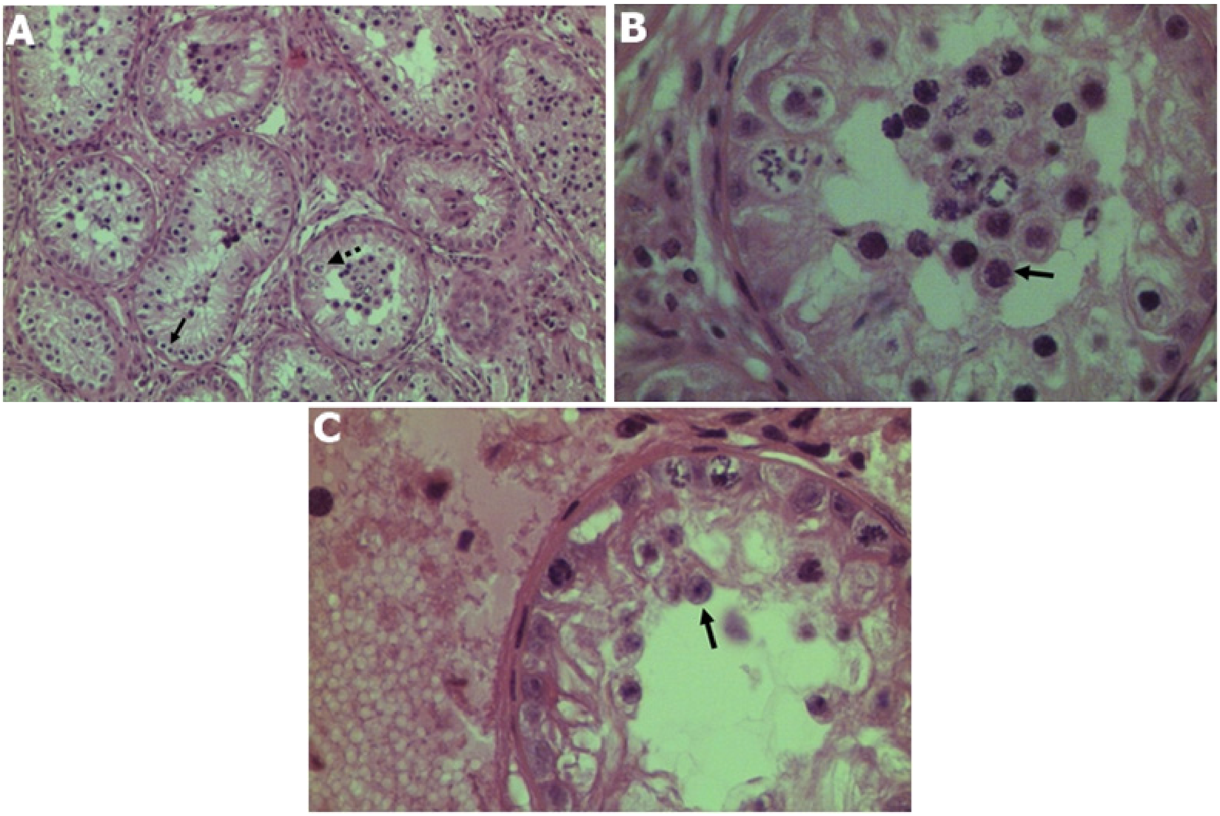

Figure 6. Photograph showing preparations from a 1.5-year-old backcross (Kropek): (A) seminiferous tubules—spermatogonia (smaller arrow) and primary spermatocyte (dotted arrow) are visible (H&E stain, 40×), (B) spermatogonia (large arrow) (H&E stain, 100×), (C) early spermatid (large arrow) (H&E stain, 100×).

was carried out several times. Later, such crossbreeding may have occurred without human intervention. When protection of the bison species became of vital importance, the problem was separation of genetically pure individuals from among the wild population. Ward et al. [15] developed a genetic test that took advantage of variation in the region of the 16S rRNA subunit in the mitochondrial genome. This test detected the presence

Table 2. Mean area of the seminiferous tubules (in μm²).

Name	Age in years	Mean area of seminiferous tubules, in μm²		
			Generation F1	Generation B1
D. cattle	1.5	34765.93		
E. bison	3.5	32743.15		
Edward	1.5		16762.91	
Dima	2.0		16648.12	
Dima	2.5		19217.85	
Kropek	1.5			16473.97

Table 3. Degree of advancement of spermatogenesis; % of seminiferous tubules in which spermatogenesis was arrested at a given stage.

Name	No. of seminiferous tubules in the field of view	Sertoli cells	Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa
D. cattle	2	5	11	14	5	64	1
E. bison	2	12	13	15	17	18	25
Edward	5	50	36	14	-	-	-
Dima	3	95	5	-	-	-	-
Dima	4	100	-	-	-	-	-
Kropek	4	54	45	1	-	-	-

of a mitochondrial genome characteristic of domestic cattle and was therefore able to pinpoint those bison that were hybrids through maternal lines of domestic cattle. Surprisingly, in our laboratory, Nowak et al. [16] showed that in over 75% of the studied population of European bison the presence of a sequence characteristic of domestic cattle was detected. Further research has shown that introgression of bovine genes to the genome of the European bison took place not through domestic cattle but rather its wild ancestor *Bos taurus*, at the time when both species sympatrically inhabited the same European areas. We can thus speculate that *Bos taurus* females sired by *Bison bonasus* males must have given birth to daughters that somehow remained in *Bison bonasus* herds. As our study has confirmed, the opposite, namely male hybrids siring offspring in bison herds, seems to be impossible due to their infertility.

Acknowledgment
This research was financed by the Ministry of Science and Higher Education of Poland under grant 2516/B/P01/2009/37.

References

1. Hayes BJ, Lewin HA, Goddard ME. The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation. Trends in Genetics 2013; 29 (4): 206-214. doi: 10.1016/j.tig.2012.11.009
2. Borghese A. Buffalo Production and Research. Rome, Italy: Food and Agriculture Organization of the United Nations; 2005. doi: 10.4081/ijas.2006.203

3. Ritz LR, Glowatzki-Mullis ML, MacHugh DE, Gaillard C. Phylogenetic analysis of the tribe Bovini using microsatellites. Animal Genetics 2000; 31: 178-185. doi: 10.1046/j.1365-2052.2000.00621.x

4. Verkaar ELC, Nijman IJ, Beeke M, Hanekamp E, Lenstra JA. Maternal and paternal lineages in cross-breeding bovine species. Has wisent a hybrid origin? Molecular Biology and Evolution 2004; 21 (7): 1165-1170. doi: 10.1093/molbev/msh064

5. Fedyk S, Sysa P. Chromosomes of European bison, domestic cattle and their hybrids. Acta Theriologica 1971; 16 (30): 465-470.

6. Graphodatsky AS, Sharshov AA, Biltueva LS, Popov VA. The karyotype of the bison (*Bison bonasus* L.). Tsitologiya i Genetika 1990; 24 (3): 34-37.

7. Gallagher DS Jr, Davis SK, De Donato M, Burzlaff JD, Womack JE et al. A molecular cytogenetic analysis of the tribe Bovini (Artiodactyla: Bovidae: Bovinae) with an emphasis on sex chromosome morphology and NOR distribution. Chromosome Research 1990; 7: 481-492. doi: 10.1023/A:1009254014526

8. Peters AHFM, Plug AW, Van Vugt MJ, De Boer P. A drying-down technique for the spreading of mammalian meiocytes from male and female germline. Chromosome Research 1997; 5: 66-71. doi: 10.1023/A:1018445520117

9. Fedyk S, Krasińska M. Studies on the spermatogenesis in European bison and domestic cattle hybrids. Acta Theriologica 1971; 16 (29): 449-464. doi: 10.4098/AT.arch.71-35

10. Krasińska M, Krasiński ZA. European Bison. The Nature Monograph. 2nd ed. Berlin, Germany: Springer-Verlag; 2013. doi: 10.1007/978-3-642-36555-3

11. Krasińska M. Progress in breeding European bison and domestic cattle hybrids and casuistics of immobilization and pasteurelosis in hybrids. Acta Theriologica 1979; 24 (15): 201-210. doi: 10.4098/AT.arch.79-20

12. Fedyk S, Krasińska M. Spermatogenesis in backcross generations of European bison and domestic cattle hybrids. Acta Theriologica 1980; 25 (15): 201-212. doi: 10.4098/AT.arch.80-15

13. Peters HF. Experimental hybridization of domestic cattle and American bison. In: 5th International Congress on Animal Reproduction and Artificial Insemination; Trento, Italy; 1964. pp. 326-332.

14. Łozicki A, Olech W, Dymnicka M, Florowski T, Adamczak L et al. Nutritive value and meat quality of domestic cattle (*Bos taurus*), zubron (*Bos taurus* × *Bison bonasus*) and European bison (*Bison bonasus*) meat. Agricultural and Food Science 2017; 26 (3): 118-128. doi: 10.23986/afsci.60516

15. Ward TJ, Bielawski JP, Davis SK, Templeton JW, Derr JN. Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap. Animal Conservation 1999; 2 (1): 51-57. doi: 10.1111/j.1469-1795.1999.tb00048.x

16. Nowak Z, Olech W. Verification of phylogenetic hypothesis concerning the evolution of genus Bison. Annals of Warsaw University of Life Sciences SGGW Animal Science 2008; 45: 65-72.