Biology of Reproduction, 2021, 104(3), 495–507
doi:10.1093/biolre/ioaa207
Review
Advance Access Publication Date: 13 November 2020

Review

Male hybrid sterility in the cattle-yak and other bovines: a review

Robert Niayale[1,2,*], Yan Cui[1,*] and Fredrick Adzitey[2]

[1]Laboratory of Animal Anatomy & Tissue Embryology, Department of Basic Veterinary Medicine, College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, Gansu Province, China and [2]Faculty of Agriculture, Animal Science Department, University for Development Studies, Tamale, Ghana

*__Correspondence:__ College of Veterinary Medicine, Gansu Agricultural University, 730070, No. 1 Yingmen Village, Anning District, Lanzhou, Gansu Province, China. E-mail: cuiyan@gsau.edu.cn/niayalerobert@gmail.com

Received 15 May 2020; Revised 16 September 2020; Editorial Decision 1 November 2020; Accepted 11 November 2020

Abstract

Hybridization is important for both animal breeders attempting to fix new phenotypic traits and researchers trying to unravel the mechanism of reproductive barriers in hybrid species and the process of speciation. In interspecies animal hybrids, gains made in terms of adaptation to environmental conditions and hybrid vigor may be offset by reduced fertility or sterility. Bovine hybrids exhibit remarkable hybrid vigor compared to their parents. However, the F1 male hybrid exhibits sterility, whereas the female is fertile. This male-biased sterility is consistent with the Haldane rule where heterogametic sex is preferentially rare, absent, or sterile in the progeny of two different species. The obstacle of fixing favorable traits and passing them to subsequent generations due to the male sterility is a major setback in improving the reproductive potential of bovines through hybridization. Multiperspective approaches such as molecular genetics, proteomics, transcriptomics, physiology, and endocrinology have been used by several researchers over the past decade in an attempt to unravel the potential mechanisms underlying male hybrid sterility. However, the mechanism of sterility in the hybrid male is still not completely unravelled. This review seeks to provide an update of the mechanisms of the sterility in the cattle-yak and other bovines.

Summary sentence

Bovine hybrids exhibit obvious hybrid vigor over either parent but while the F1 female is fertile, the male exhibits sterility. This prohibits the utilization of the hybrid vigor hence poses a challenge to the improvement of local species through hybridization.

Key words: cattle-yak, bovines, hybrid, sterility, genes, heterosis, methylation.

Introduction

Crossbreeding is practiced in an attempt to fix desirable traits in subsequent generations. Hybridization in bovines such as cattle-yak (*Bos taurus* × yak), cattalo (domestic cattle × American bison), Yakalo (Tibetan yak × buffalo), Zubron (European bison × domestic cattle) is practiced by farmers with the sole aim of improving the reproductive performance. Crossbreeding is used to improve the performance of animals especially under harsh conditions [1]. In the cold, and hypoxic high altitude Qinghai–Tibetan plateau of China, the hybridization is usually done between the yak and the domestic cattle (*Bos taurus*) to harness the positive traits of the two species without compromising the resistance to the harsh environment. Yak females crossed with bulls of local cattle is regarded as the normal hybridization and the F1 hybrid is called "true Pian Niu" [2] or cattle-yak in English, whereas the reciprocal cross between yak bull and female cattle is considered as "counter-hybridization" and the F1 progeny are called "false Pian Niu." Although hybridization in bovines results in heterosis, the male hybrids are often partially or completely sterile, whereas the females are fertile. Practically, backcrossing upgrading

systems have been practiced between fertile hybrid females and the fertile male parents over generations to dilute the sterility in the males. However, even though sometimes successful, the adaptability of the offspring declines and hybrid vigor disappears with increasing hybridization generation. Members of the subfamily Bovinae (*Bison bison*, *Bison bonasus*, *Bubalis bubalis*, *Bos taurus*, and *Bos indicus*) diverged many years ago but have a similar diploid number of chromosomes (2n = 60), consisting 58 acrocentric autosomes except for sex chromosomes where subtle differences in morphology exist [3, 4]. Therefore, hybridization in these species is possible, but as indicated, the hybrid is often sterile. This is a setback in attempts to fix desirable features in these animals through hybridization, hence poses a challenge in improvement of the purebreds. The objective of this review is to provide an update and significant insight into the mechanism underlying the male hybrid sterility in the cattle-yak and some other bovine hybrids. This will give readers a current update on research on bovine hybrid sterility and help identify knowledge gaps for future research purposes geared toward addressing the phenomenon.

Heterosis in bovine hybrids

Hybridization among bovine species occurs either in the wild (natural) or in a controlled environment under captivity, and the hybrids often exhibit obvious hybrid vigor over either parent (Table 1). For instance, the *Bos taurus* and yak hybrid retain the characteristics of both parents by inheriting the adaptability to the harsh high-altitude environments from the yak and high productivity from the *Bos taurus* [5]. However, the male hybrid is most often sterile, making the passing of the desirable traits to subsequent generations impossible. In the cattle-yak, although the male hybrids have normal external genitalia, libido, and mating behavior, matured sperm is absent in the seminal fluid. Also, the hybrids and backcross generations are less adapted to the harsh conditions such as high altitudes and cold temperatures, whereas others have reduced heterosis and require improved management to survive [2, 6]. Hence, the male hybrids are slaughtered for meat or used as teaser bulls, making hybridization, and backcrossing commercially unattractive.

Causes of male hybrid sterility

Hybrid male sterility (HMS) is a postzygotic reproductive isolation mechanism that prevents the successful formation of fertile offspring after fertilization between different species [7]. This phenomenon occurs during the early stages of speciation, which restricts gene flow between closely related, sexually reproducing organisms. HMS is a universal phenomenon observed in many interspecies hybrids of eukaryotes, ranging from yeast, plants, insects, birds to mammals [10, 11]. Interspecific hybridization may either result in total or partial sterility of the hybrids, hybrid inviability, or a reduction in viability or fertility in backcrosses either in one or both sexes. In mammals, HMS affects heterogametic sex (males) but not homogametic sex (females), consistent with the Haldane rule [12]. Although studies are still on-going to unravelled the cause of HMS, numerical and structural differences in chromosomes resulting in incompatible interaction between parental genes loci, aberrations in meiosis, defective expression of testis-specific coding, and noncoding genes, and transcriptional regulation have been implicated. The common consequence of these causes is a disruption in spermatogenesis in F1 hybrids, characterized by reduced testis size, spermatogenic arrest, reduced germ cell numbers, and absence of matured spermatozoa and [13]. Chromosomal and genic incompatibility are the two common causes of postzygote isolation, but incompatibilities due to deleterious interactions among genes are considered the primary cause of hybrid inviability and sterility in most animals [10, 14, 15]. This has become known as the Dobzhansky–Muller model, which proposes the functional divergence between interacting loci in different lineages yields incompatible interactions in their hybrids resulting in sterility. In bovine hybrids such as the F1 progeny of European Bison and domestic cattle, the HMS was attributed to abnormal conjugation resulting from lack of a sufficient homology between chromosomes from the parents or due to disturbances in the divisional spindle formation in spermatocytes [16]. This suggestion is consistent with observations by Peters [17] who observed the presence of univalents at meiotic metaphase cells in hybrids, suggesting chromosomal incompatibility. The disproportionately large contribution of the X chromosome to hybrid male sterility, referred to as the "Large X effect," with the upregulation of X chromosome transcripts in testes of sterile hybrids relative to that of the autosomes has also been implicated [18–20]. Two mechanisms have been proposed to cause the large X effect. First is the "Dominance theory," premised on the exposure of recessive epistatic X-linked incompatibilities in hemizygous males [21]. Also, the "Faster-male theory" proposes that male-limited reproductive traits accumulate faster than female-limited traits, either due to sexual selection or the high sensitivity of spermatogenesis to molecular disturbances [22]. Furthermore, the failure of meiotic sex chromosome inactivation (MSCI) where genes on sex chromosomes are inactivated during meiosis contributes to HMS [23]. Cells with defective MSCI cause meiotic arrest and these defective cells are eliminated by late pachytene resulting in complete sterility [24, 25]. In a cross between *Mus musculus musculus* and *M. m. domesticus*, a large number of X-linked genes were overexpressed in the testes of the sterile F1 males [26, 27]. This suggests that MSCI is defective in the X chromosome of the sterile male hybrid; hence the expression of genes would have otherwise been silenced. Other studies attribute the HMS in the F1 male to X–Y or X-autosome incompatibilities, or a combination of both. In dwarf hamsters, the HMS was as a result of the dissociation between the X and Y chromosomes, resulting in X–Y asynapsis and meiotic arrest [28, 29] The meiotic arrest of the cells with X–Y dissociation could be a result of meiotic silencing of unsynapsed chromatin (MSUC) spreading to the pseudoautosomal region (PAR) resulting in the inactivation of genes essential for spermatogenesis. Also, the lack of homology at the PAR due to the divergence between parental species correlates with X–Y dissociation [30, 31]. The PAR shows a higher rate of evolution than autosomal or X chromosome-specific genes [32, 33]. In the cattle-yak, meiotic arrest at early prophase I is reported to be the cause of the HMS, which triggers germ cell apoptosis at pachytene stage resulting in absence of matured spermatozoa in the epididymis [34]. In hybrids from European bison × cattle, chromosome pairing abnormalities leads cessation of spermatogenesis at spermatogonial or primary spermatocyte stage [8, 16]. American bison × cattle hybrids also showed a considerable percentage of cells with an abnormal number of bivalents [35]. Because of these abnormalities, F1 hybrids usually exhibit meiotic arrest, and the defective meiocytes eliminated via apoptosis, resulting in azoospermia and severe degeneration of the seminiferous tubules. Even though the male hybrids are sterile, they exhibit normal secondary sexual traits like testis development, libido, and ability to mating. However, MCSI, MSUC, and the large X effect are yet to be determine in bovine hybrids, hence will be focus for future studies. Also, marking of the distribution

Table 1. Hybrid vigor of some selected bovine hybrids.

Hybrid	Parents		Sex afflicted by sterility	Advantage(s)	Reference
	Sire (♂)	Dam (♀)			
Cattle-yak (2n = 60)	Cattle	Yak	Male	Taller body frame, robust, faster growth rate, drought-tolerant, more resistant to diseases, high production of milk and meat, easy to tame	[2, 6, 95, 96]
Yakalo (2n = 60)	Tibetan yak	buffalo	Male	Ability to tolerate harsh winter conditions	[97, 98]
Cattalo (2n = 60)	Domestic cattle	Buffalo	Male (rarely fertile)	More docile, tolerant to cold and heat, nonselective grazers, better calf feed to gain ratio. Meat is more tender and nutritious	[98–100]
Zubron (2n = 60)	European Bison	Domestic cattle	Male	High nutritive value and quality of the meat, Hardier and heavier, more resistant to diseases and pest	[8, 9, 98, 101]
Yattle (2n = 60)	Yak	Domestic cattle	Male	Larger body frame, hardy, more robust, require no additional supplements better survivability, high production of milk and meat with low fat and cholesterol	[102, 103]

recombination hotspot and positions of the double-strand breaks (DSBs) by PRDM9, the generation of DSBs by Spo11, synapsis, and recombination in the cattle-yak male hybrid has not been determined yet. Studies in these areas would help determine if the root cause of HMS in bovines hybrids is a failure of pairing and synapsis and their secondary effects on fertility.

Histological analysis of testis of bovine hybrids and backcrosses

Abnormalities with gene expression and regulation usually manifest in changes in histology and cellular constitution of the tissue affected. Histological analysis of bovine hybrid testis revealed phenotypes similar to that of other mammalian interspecies hybrids such as equines, murine, and felines. In the cattle-yak, the seminiferous tubules consist of spermatogonia and a few spermatocytes but are devoid of late-stage germ cells unlike the male parents, which had all types of germ cells ranging from spermatogonia to spermatozoa [36–39]. Figure 1 shows a comparison of the histological sections of domestic cattle, yak, and cattle-yak from our on-going research. In the F1 progeny of European bison and domestic cattle, only spermatogonia and Sertoli cells were observed in the tubules of F1 hybrids between, with the seminiferous tubules of the F1 hybrid being loosely arranged and neighboring tubules are not in contact through their basement membranes [8]. The membranes of the tubules and walls of the blood vessels were also thickened. This ultimately results in absence of germ cells beyond the spermatocyte stage, possibly due to their depletion via apoptosis. However, the situation seems to improve in backcross generations. The male hybrid cattle-yak in B3 generation could produce sperm, and the tubule structure, interstitial tissue thickness, and blood vessel density improved [2, 40]. Życzyński et al. [9] also found completed spermatogenesis in B2 and B3 backcross generations with all stages of spermatogenesis detected with a greater percentage of cattle genes in the backcrosses. In the cross between European bison and domestic cattle, some B2 and all B3 generations had complete spermatogenesis, but the B1 and some B2 backcrosses had many cases of degeneration of interstitial tissue, hypertrophy of connective tissue, and arrested spermatogenesis [8, 16]. The B2 generations either continues to its normal termination or ceases at the spermatogonia stage, but the thickening of the walls of capillary vessels observed in many F1 and B1 hybrids did not occur at all in B2 and B3 generations. The depletion of the germ cells often results in reduced testis weight, decreased tubule surface area, thinning of the germinal epithelium, and widening of the tubule lumen area. A reduction in testis weight, thinning of the germinal epithelium, and widening of the tubule lumen area caused by depletion of germ cells was observed in the cattle-yak tubule section [38] and our study. The hybrid (Holstein–Friesian × Tharparkar) had a wider seminiferous tubule area and significantly lower Sertoli cells to spermatogenic cells ratio compared to Holstein–Friesian purebred [41]. Taken together, tubules of hybrid bovines lack late stage germ cells such as spermatids and spermatozoa, possibly because of their depletion via apoptosis triggered by arrest in meiosis, but this phenotype improves with increasing backcross generations.

Backcrossing with successive generations dilutes the hybrid sterility

Backcrossing of fertile hybrid females with either of the fertile parental lines has been practiced in an attempt to establish fertile males. There was a gradual recovery of sperm production capacities in the male hybrid with an increased in backcross generations (3rd or 4th) with a recovery of normal homologous chromosome pairing during meiosis [2, 5, 42]. This suggests that the loss of homologous pairing and other related processes is the cause for the lack of sperm in the hybrids. There was also gradual improvement in the development of testis tissue and a reduction in the death rate of spermatogonia in the offspring. The backcross generations (B2 and B3) between hybrid European bison × cattle females to either of the fertile males resulted in complete spermatogenesis in

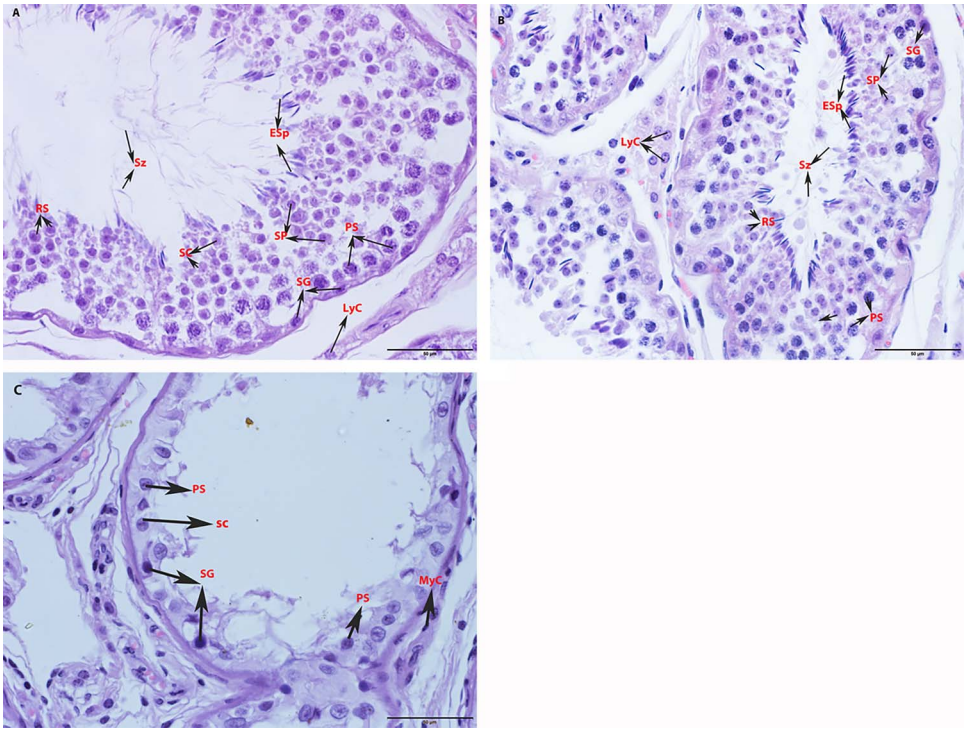

Figure 1. H&E staining of testicular samples of cattle-yak and its parents. A: Yak B: Cattle C: Cattle-yak. MyC-Myoid cell, SG-Spermatogonia, PS-Primary spermatocyte, SP-Secondary spermatocyte, RS-Round spermatid, EPs-Elongating Spermatid, Sz-Spermatozoa, Author's own data.

the backcross, even though progeny were not obtained from these males from natural mating [8, 16]. All stages of spermatogenesis were frequently detected with a greater percentage of cattle genes in the backcrosses. It was possible to obtain several fertile males in B2 backcross generation of American bison and domestic cattle when F1 females were backcrossed with the fertile males [35]. Taken together, sterility in the male hybrids becomes diluted with increasing consecutive backcrossing due to correction of meiotic processes, which appeared defective in the hybrids. However, the backcrosses exhibit no heterosis and have a low adaptation to the harsh environmental conditions, hence have little agricultural value and not worth investing time and resources.

Studies on the mechanism of male hybrid sterility in bovines

Studies have attributed the HMS to failure to properly execute spermatogenesis. Several studies use multiperspective approaches such as cytogenetics, histology, endocrinology, and molecular genetics to understand the mechanism of hybrid male sterility in bovines.

Morphological and numerical differences in chromosomes

The sterility of male hybrids was initially sought on the chromosomal level because differences in chromosome number and structure between interspecific hybrids inherited from both parents often result in sterility. A variation in parental chromosome structure often results in asynapsis/incomplete synapsis, and subsequent aberrant meiotic recombination resulting in meiotic arrest and gamete failure [43]. The yak, domestic cattle, and their hybrids have the same diploid number of chromosomes (2n = 60), consisting of 29 acrocentric autosomes and the X chromosome being submetacentric, whereas the Y chromosome in the yak and the hybrid is submetacentric and metacentric in the ox [5, 44–46]. Also, no remarkable difference was observed in G- and C-banding as well as in relative chromosomal length. In the bison (*Bison bonasus* L.), all the autosomes and the X-chromosome were similar to corresponding chromosomes of *Bos taurus*, whereas the Y-chromosome is acrocentric. Hybrids between the bison (*Bison bison*) and the domestic cattle (*Bos taurus*) and between *Bos banteng* and *B. taurus* not only have the same diploid number (2n = 60) but also have an indistinguishable G-banding pattern with the parents [47], yet the F1 hybrids are sterile. A comparison of the Y chromosome morphology of some bovine species containing 2n = 60 (*Bison bison*, *Bison bonasus*, *Bos taurus*, and *Bos indicus*) revealed insufficient structural differences between Y chromosomes of these species to explain the sterility of male hybrids [16]. As stated earlier, there is a gradual restoration in the male hybrids when the fertile females are backcrossed with males of either parental line. This suggests that interspecific bovine hybrids exhibit sterility despite inheriting morphologically identical sets of chromosomes from both parents. Thus, the morphological and numerical difference in chromosomes from the parental lines is not the cause for the failure to complete spermatogenesis accurately, the cause of the sterility in the cattle-yak.

Endocrine and hormonal factors

Gonadal hormones (follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play pivotal roles in regulating gametogenesis and other aspects of the male reproductive system. Therefore, abnormalities in the production or level of production of these hormones may impact fertility negatively. A study observed the distal part

of the anterior pituitary of the male cattle-yak and yak revealed an expansion in FSH cell of the cattle-yak, a malformation of the cell nucleus, the entering of plasma into the nucleus, as well as the presence of little secretory granule compared to the yak [48]. This resulted in reduced production of FSH in the hybrids and was initially thought as the reason for the male sterility in the cattle-yak. However, shortly this finding, two studies [49, 50] revealed that the F1 male cattle-yak pituitary secretion and blood levels of FSH and LH were normal and consistent with that of the parents. Thus, refutes the earlier claim of variation in hormone production as the direct cause of the male cattle-yak sterility. Also, frequently intravenously injecting well-fed crossbred calves with FSH and LH did not result in sperm production after 12 months, except an increase in libido [51]. Fedyk and Krasińsk [16] also observed degeneration and overgrowth of the interstitial cells with connective tissue, which is certainly related to the lack of stimulatory activity of hormones. These findings suggest that abnormal hormone secretion may not be the mechanism underlying the sterility in the male cattle-yak.

Genetic and epigenetic factors

Since the species in the subfamily Bovinae have similar chromosome number and structure, the sterility may come from, mutation, inactivation, or misexpression of some genes involved in spermatogenesis. Several studies focused on the comparative expression of candidate genes and their methylation levels in the testis of the hybrids and their male parents. These selected genes have been identified in other organisms to be involved in spermatogenesis, germ cell development, and sterility.

Expression of genes involved in spermatogenesis

Spermatogenesis is a highly ordered and genetically coordinated process involving numerous coding and their protein products as well as noncoding genes. The events occurring during spermatogenesis are controlled by well-synchronized gene regulation at the transcriptional, posttranscriptional, and epigenetic levels [52, 53]. Central to spermatogenesis is the key step of meiosis and defects in genes that control various stages of meiosis often lead to male infertility or sterility. Aberrations in the expression of these genes and their protein products result in sterility. The genes involved in spermatogenesis whose mutations or deficiency have been called into play in the pathogenesis of the male sterility are shown in Table 2. Some of the genes play crucial roles in spermatogenesis, such as cell proliferation, differentiation, apoptosis, cell transformation, meiosis (DSB processing, synapsis, pairing, DSB repair, recombination, and cross-over formation), whereas others play in (*CDC2*, *CDC25A*, *DAZ*, *BOULE*, *DMC1*, *SNRPN*, *DMRT7*, *DDX4*, *SYCP1*, *SYCP2*, *SYCP3*, *FK506*, and *MEI1*). Also, some of the genes function in ensuring genome stability by regulating transposon (*PIWIL1*), whereas others regulate apoptosis and protect the germ cells during stress (*HSP 27*, *70& 90*, *p53*). As expected, the expression of protamine (*PRM1* and *PRM2*) and *LDHC*, which are expressed in spermatids and spermatozoa, respectively, was low in the hybrid male, possibly due to the inability of spermatogenesis in the cattle-yak to reach these advanced stages.

The low mRNA expression in some of the genes was attributed to the higher methylation level of their promoter region in the cattle-yak. For instance, high methylation was found in *b-SYCP3* (yak, 81.58% vs. cattle-yak 86.84%; $p > 0.05$), *FKBP6* (yak, 60% vs. cattle-yak, 85%; $p < 0.01$), and *DAZL* (cattle-yak (85.6%), cattle 69.8%, and yaks 71.4%). DNA methylation is one of the important epigenetic modifications, which play a role in genomic imprinting, transposon silencing, X-chromosome inactivation, and regulating tissue-specific gene expression during mammalian development [54]. Because of its influence on chromatin structure and DNA conformation, it regulates gene expression and affects protein function. Hence, hyper- or hypo-methylation of important genes involved in spermatogenesis affects fertility by repressing their expression, suggesting that the high methylation of some genes affects their transcription, hence the low mRNA expression observed.

Expression of histone methyltransferases

Before the generation of double breaks to initiate meiosis recombination in mammals, *PRDM9* (PR domain containing 9) plays a key role in recombination hotspot specification and directs the positions of the DSBs events that initiate meiotic in both humans and mice [55, 56]. PRDM9 is a zinc finger protein that binds DNA at specific locations in the genome where it tri-methylate histone H3 at lysine 4 (H3K4me3) and 36 (H3K36me3) at surrounding nucleosomes [57]. *PRDM9* is expressed exclusively in germ cells of both males and females and plays a role in histone methylation, resulting in the aberrant expression of several interacting genes in sterile hybrid males [56, 58]. There was a significant decrease in *PRDM9* mRNA expression levels in the testis of the male cattle-yak and sexually immature yak calves compared to the adult yaks [40, 59]. In mice, the deficiency of *PRDM9* resulted in meiotic-arrest at mid-prophase I is manifested by defective DSB repair as well as incomplete synapsis of homologous chromosome [58, 60]. This suggests that the low mRNA expression of PRDM9 could decrease its histone methyltransferase activity essential for meiotic DNA DSB formation at its binding sites resulting in insufficient generation of DSBs at the onset of meiosis, leading to the aberrant marking of the position of the DSBs. Male (PWD × B6)F1PWD$^{/B6}$ mouse hybrids showed complete sterility characterized by failures to sire pups, form sex body, and synapsis [61]. However, these defects were completely rescued and the sterility reversed in (PWD × B6)F1$^{PWD/H}$ hybrids, which inherited an engineered humanized ZF-array showing an increase in symmetric binding, restoration of proper synapsis, and fertility. This finding suggests that the ZF domain of PRDM9 and likely the PRDM9 protein DNA-binding properties underlies the role of PRDM9 in hybrid sterility. The formation, distribution, and the protein DNA-binding properties of PRDM9 have not been studied in the cattle-yak. In cattle, the number and sequence of *PRDM9* zinc finger exhibit great variations among different cattle breeds and within the cattle family and is associated with recombination rates [62, 63]. Histone modification, such as methylation, is essential for normal spermatogenesis, as it regulates timely and cell-specific expression of genes, whereas the methylation of some histones (H3K4me1/2/3, H4K20me1, and H4K20me2) is associated with enhancer region and transcription activation, some (H3K9/27 and H4K20me3) label gene repression [64–67]. A recent study revealed that the morphology of gonocytes was normal and was capable of entering meiosis in yak and cattle-yak [59]. However, H3K27me3 and H4K20me3, which are involved in gene repression, were enriched in the gonocytes of cattle-yak, suggesting that although the gonocyte population was established in cattle-yak, epigenetic programs directing gene expression were aberrant in gonocytes and could indirectly cause defects in meiosis of spermatocytes. Also, H3K4me3 was depleted, whereas H3K27me3 and H4K20me3 were enriched in Sertoli cells. The authors concluded that gene activation machinery was repressed, whereas gene silencing program was enhanced in Sertoli cells of

Table 2. Candidate genes and their mRNA expression and methylation levels.

Gene category/function	Gene symbol	Expression level	Methylation level	Reference
DAZ family of genes (regulate the translation and transport of key transcripts in germ cells)	*DAZL*	Not detected	n/a	[104]
	BOULE	Down	Hypermethylated	[34, 105]
	DAZL	Down	Hypermethylated	[37, 106]
Synaptonemal complex genes (lateral and central element	*SYCP2*	Down	n/a	[107]
	SYCP3	Down	Higher	[108, 109]
	FKBP6	Down	Hypermethylated	[110, 111]
Genes regulating germ cell division cycle/development	*CDC2* and *CDC25A*	Down	n/a	[112]
	DDX4	Down	Hypermethylated	[113]
DM domain family of gene (function in histone modifications that maintains transcriptional silencing of the sex chromosomes)	*DMRT7*	Down	n/a	[39, 114]
Recombinases (strand exchange, DSB repair and Meiotic recombination)	*RAD51*	Down	n/a	Niayale et al. (Unpublished)
	DMC1	Down	n/a	[115]
	MEI1	Down	Hypermethylated	[116]
Pre-mRNA processing e.g. tissue-specific alternative splicing events	*SNRPN*	Down	Hypermethylated	[117]
Transposon regulator (suppress retrotransposons and protects the integrity of the genome of germ cells	*PIWIL1*	Down	Hypermethylated	[118]
Imprinted genes	*IGF2*	Down	Hypermethylated (ns)	[119]
	H19	Up	Hypo-methylated	[69]
Histone methyltransferases	*EHMT2*	Up	n/a	[59]
	MLL5	up	n/a	[59]
	SUV420H1	Down	n/a	[59]
	PRDM9	Down	n/a	[40] [59]
Genes expressed in spermatid/sperm (DNA compaction and sperm energy generation)	*PRM1* and *PRM2*	Down	n/a	[120]
	LDHC	Down	n/a	[121]
Y-transitional/Y-ampliconic region genes	*TSPY-T2*, *HSPY*, *ZNF280BY* *PRAMEY-T2*	Down	n/a	[122]
Molecular chaperones (help in protecting cells during stress and maintenance of cellular integrity and viability)	*HSP70/90*	Hsp70 down, Hsp90 up	n/a	[72]
	HSP27/P53	Hsp27 down, p53 up	n/a	[73]

n/a = not available; ns = not significant.

cattle-yak. Since Sertoli cells support the differentiation of spermatogonia, meiosis progression, and spermiogenesis [68], the authors surmised that the dysregulation of gene expression in Sertoli cells probably led to the formation of an environment not conducive for meiosis hence caused massive germ cell loss and sterility in the cattle-yak. Also, at 2 years, relative concentrations of *MLL5* and *SUV420H1* increased, whereas *PRDM9* and *EHMT2* decreased in cattle-yaks compared to those of yak testes (Figure 2).

Expression of imprinted genes (*IGF2* and *H19*)

Insulin growth factor (*IGF2*) is a paternally expressed and maternally imprinted gene involved in cell proliferation, differentiation, and apoptosis, whose expression depends on the methylation status of *H19*. The expression of *IGF2* in the testes of cattle yaks was significantly lower than the parents with a highly methylated DMR in the cattle-yak, yaks, and cattle (Figure 2). In the paternal allele, the ICR of *H19* is methylated, which prevents CCCTC-binding factor from binding and disrupts insulator function, hence allow the downstream enhancers to activate paternal *IGF2* expression but silence *H19* expression. The methylation level of the CTCF-binding sites in *H19* ICR was significantly lower in cattle-yak than both parents (70, 59.10, and 48.82% in cattle, yak, and cattle-yak, respectively), resulting in significantly higher expression *H19* mRNA in cattle-yak [69]. *H19* gene is controlled by the methylation status of its ICR [70]. The hypo-methylation of *H19* suggests that

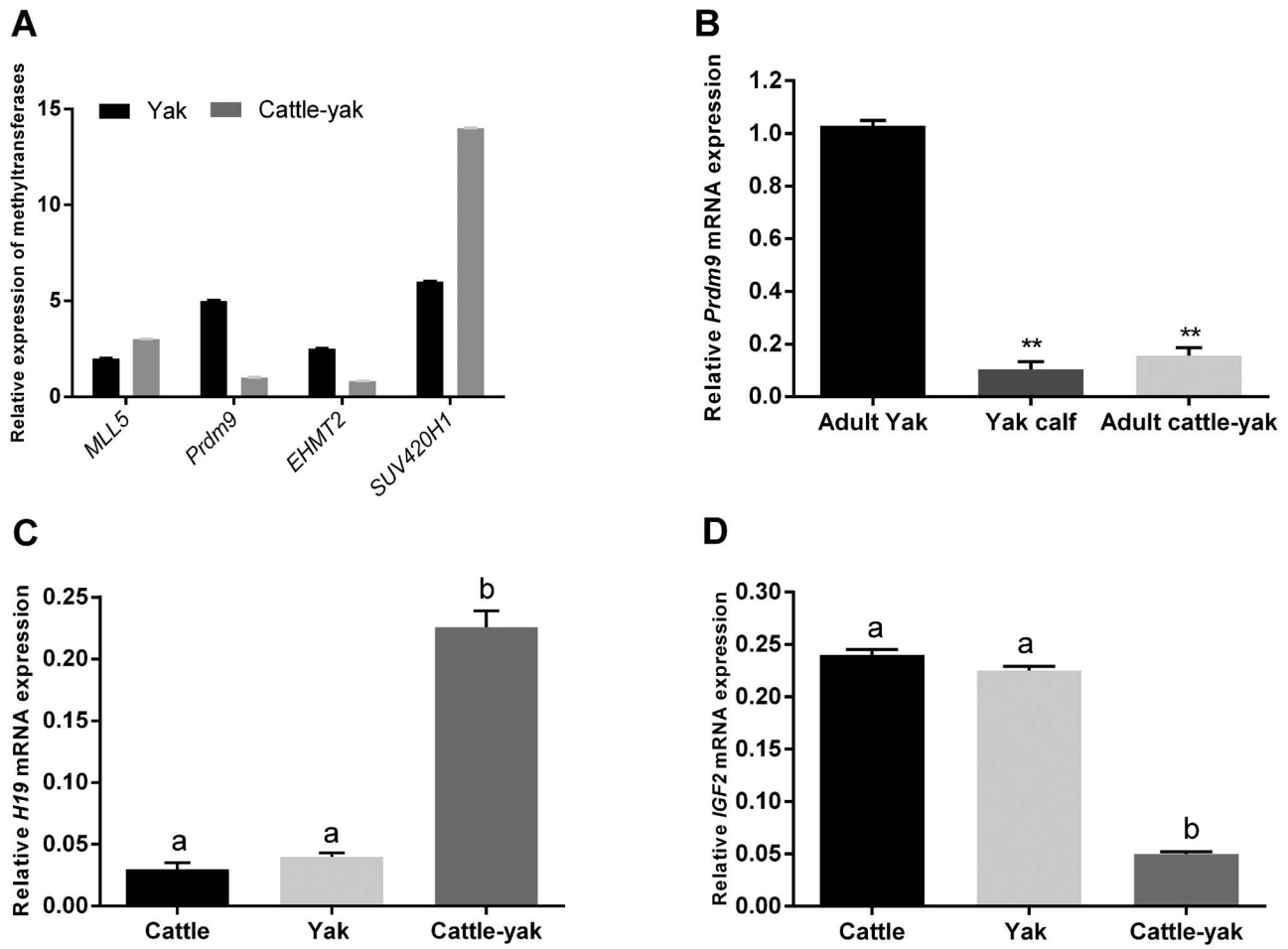

Figure 2. Comparative mRNA expression of methyl transferases and imprinted genes. A: relative expression levels *MLL5, PRDM9, EHMT2* and *SUV420H1* transcripts in testes of 2 year old yak and cattle-yak testes. Adopted and modified from [59]. B: Relative *Prdm9* mRNA levels in testes of adult yak, yak calf and cattle-yak (Adopted and modified from [40]. C: relative expression levels of the *H19* gene in testes of cattle, yak and cattle-yak. Adopted and modified from [119]. D: Comparisons of relative *IGF2* mRNA levels in testes of adult yak, yak calf and cattle-yak. Adopted and modified from [69]. Different letters represent a significant difference ($p<0.05$), $^{**}p<0.001$.

CTCF was allowed to bind to downstream enhancers to deactivate paternal *IGF2* expression and enhanced *H19* expression, resulting in disruption of germ cell proliferation, differentiation, and increased depletion of germ cells via apoptosis as seen in the hybrids. A knowledge gap identified here is to determine imprinting problems such as fetal and placental growth in cattle-yak and other bovine hybrids since imprinting generally relates to fetal and maternal conflict and defects in imprinting affects the growth regulation of the embryo and the placenta. This would be considered for future study.

Expression of molecular chaperones (heat shock proteins)

Molecular chaperones such as heat shock proteins (HSPs) play a role in gametogenesis by protecting testicular development and spermatogenesis [71], especially under stressful conditions. They regulate germ cell apoptosis when the cell is subjected to stressful conditions such as heat and hypoxia (conditions that pertain in the cattle-yak's habitat). The mRNA expression of *HSP 27* and *70* was found to be low, whereas the mRNA and protein expression of *HSP90* and *p53* were found to be higher in the cattle-yak compared to the yak [72, 73]. The HSP70/90 proteins were located in the epithelial, spermatogenic, and mesenchymal cells. The authors hypothesized that the high expression of *HSP90* coupled with *p53* causes the blockage of spermatocyte development by enhancing germ cell apoptosis in the cattle-yak testis. Previous studies in mice indicated that inhibiting *HSP90* expression and a decrease in *p53* expression could protect and restore the damaged reproductive function by reducing apoptosis [74, 75], suggesting that *p53* plays a role in regulating the effect of HSP90 on the testis.

Testis transcriptome analysis cattle-yak and its male parents

Transcriptome analysis of testicular tissue is used to discover differentially expressed genes between hybrids and their parental species, which may give insight into the possible cause of the hybrid sterility. These studies help to reveal the upregulation and downregulation of genes that regulate spermatogenesis.

The differential expression of genes in the cattle-yak is shown in Figure 3. In the cattle-yak, synaptonemal complex (SC)-related genes (*FKBP6*, *SYCE3*, and *DMRT7*), genes involved in DSBs and

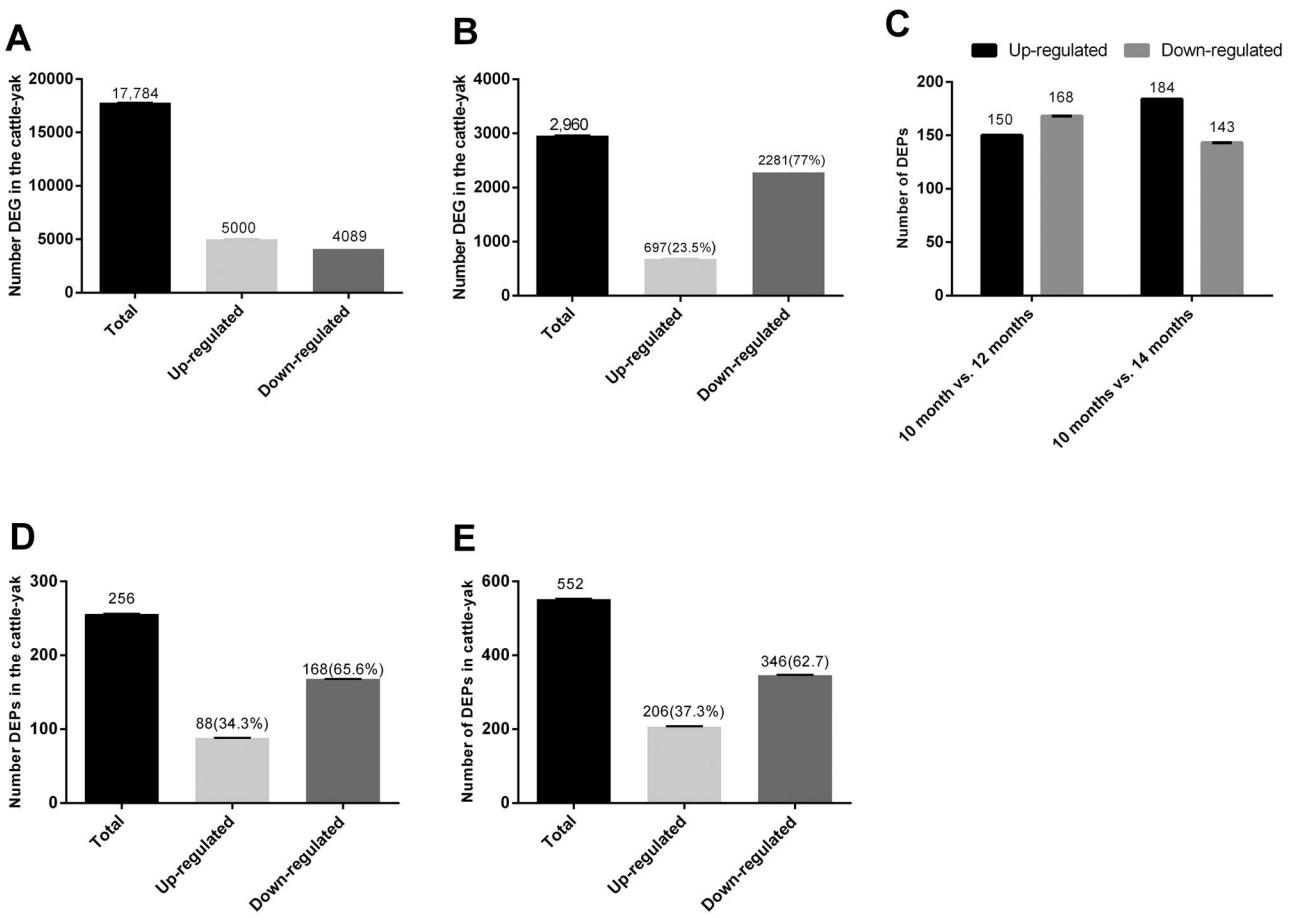

Figure 3. Differential expression genes and proteins in the cattle-yak. A: Number of DEGs in the cattle-yak compared to the yak Adopted and modified from [92]. B: Number of DEGs in the cattle-yak compared to the yak (Adopted and modified from [76]). C: Number of DEP of 10 months vs. 12 months and 10 months vs. 14 months cattle-yaks (Adopted and modified from [83]. D: Total, upregulated, down-regulated proteins in the cattle-yak testis (Adopted and modified from [123]. E: Total, up-regulated, down-regulated proteins in the cattle-yak testis (Adopted and modified from [124].

homologous repair processes (*Spo11* and *Dmc1*), and pro-apoptosis genes (*BAX*, *CAS-3*, *6 &7*, *p53*, *TNF-A*, *TRAIL*, and *BMP8B*) were among the upregulated genes, whereas anti-apoptosis genes such as *BCL-2* and *Survivin* were downregulated. This finding suggests that the downregulation of these genes may lead to the impaired SC assembly, aberrant homologous pairing, and synapsis, as well as increased apoptosis observed in the cattle-yak resulting in the HMS. Cai et al. [36] reported the upregulation of *STRA8* and *NLRP14* may be involved in the accumulation of undifferentiated spermatogonia and severe cell death in the cattle-yak. Also, genes associated with cell cycle progression and genome integrity of spermatogonia (*PIWIL1* and *SPP1*) and genes involved in meiosis (*SPIN2B*, *CDKN2C*, *CYP26A1*, *TSSK1B*, *GGN*, *MAK*, *INSL6*, *RNF212*, *TSSK2*, *OVOL1*, and *TSSK6*) were downregulated. In summary, they hypothesized that the arrest in spermatogenesis in the cattle-yak possibly occurred at the stage of spermatogonial differentiation and gets intensified during meiosis, which led to a remarkably reduced number of sperms with morphological abnormalities exhibiting an inability to fertilize eggs. This requires further studies on single-cell RNA sequencing to reveal which gene is expressed in which cell type and at what stage to understand the stage of germ arrest in the cattle-yak.

Analysis of testis proteomes of cattle-yak and its male parents

Proteomics approach identifies all the proteins expressed in an organism, organ tissue, or cell under different physiological contexts and is used to complement transcriptomic techniques. The result of testis proteome of cattle-yak is shown in Figure 3. Most of the down-regulated proteins in cattle-yak are involved in defects in various metabolic pathways and cellular processes during spermatogenesis such as extracellular matrix organization, germ cell development, and response to stimulus.

Expression of noncoding RNAs in the testis of cattle-yak

Noncoding RNAs (~19–24 nt in length) including microRNAs (miRNAs), small interfering RNAs, PIWI-interacting RNAs, and long noncoding RNAs (LncRNAs) are essentially involved in the posttranscriptional regulations of gene expression during spermatogenesis [76, 77]. The differential expression of noncoding RNAs in yak and cattle-yak is shown in Figure 4. The results revealed that the expression of all the six known miRNAs (*bta-miRNA-449a/b*,

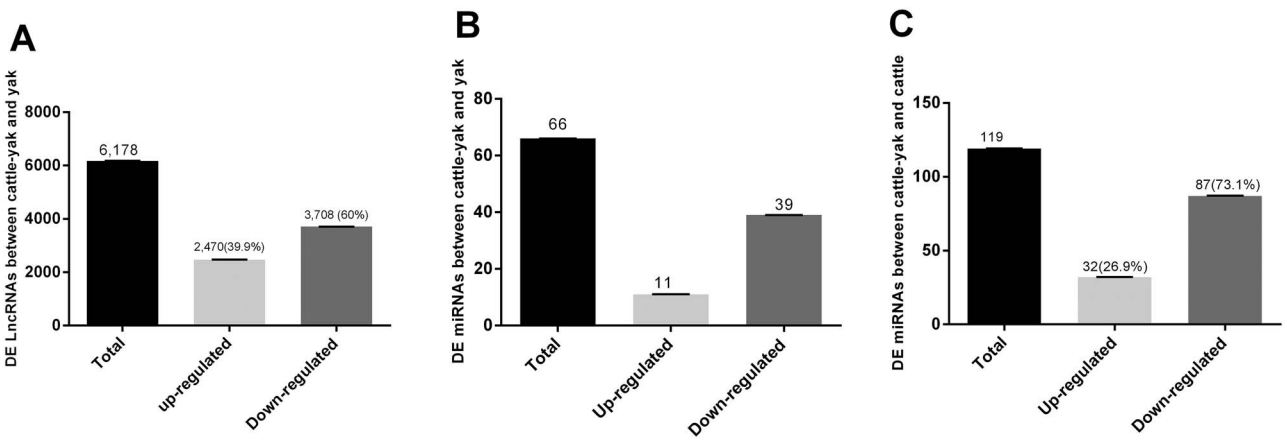

Figure 4. Comparative expression of non-coding RNAs in the cattle-yak compared to its parents A: Differential expression of LncRNAs between the cattle-yak and yak (Adopted and modified from [125]. B: Differential expression of miRNAs between the cattle-yak and yak. Adopted and modified from [79]. C: Differential expression of miRNAs between the cattle-yak and cattle. Adopted and modified from [126].

bta-miRNA-135, *bta-miRNA-19b*, *bta-miRNA-378* and *bta-miRNA-184*) and two novel miRNAs (*bta-novel 10* and *bta-novel 11*) were downregulated in cattle-yak [78]. Considering the function of these miRNAs, the downregulation of (bta-miR-135a, bta-miR-378, and bta-miR-184) may cause a reduction in the population of spermatogenic stem cells (SSCs) and cause aberrations in spermatogenesis. Also, the downregulation of bta-miR-34b/c and bta-miR-449 clusters could repress the expression of *NANOS3*, *SYCP3*, and *STRA8*, resulting in mitosis-to-meiosis transition failure and contribute to the spermatogenic arrest observed in the cattle-yak. LncRNAs moderate the transcriptional status of individual mRNA genes or the entire chromosome [79]. Some of the LncRNA-mediated target genes (*IGF2*, *PTGDS*, *GLIS3*, *MEST*, *NTOCH2*, *HOXA10*, and *HOXA11*) were associated with male infertility cattle-yak. In conclusion, the downregulated noncoding RNAs may contribute to the silencing of key genes that play crucial roles in spermatogenesis resulting in sterility in the hybrid.

Integrating the molecular genetics and histological data

Spermatogenesis is a unique process that results in halving of the DNA content the generation of terminally differentiated spermatozoa capable of delivering the genome of the male parent to the oocyte for subsequent development to yield an offspring. Because of HMS in interspecific hybrids, several studies investigated the mechanisms underlying the phenomenon. Comparative studies of the number and morphology of the karyotype, expression of genes associated with meiosis between the bovine hybrids and their parents as well as the impact of these on the testicular histological have been done. In the subfamily Bovinae, the chromosome number ($2n = 60$) and morphology were found to be similar [3–5, 44, 46], hence insufficient to cause pairing abnormalities to lead to HMS. Therefore, focus then shifted to molecular mechanisms that cause hybrid sterility. The process of spermatogenesis is under stringent genetic control involving numerous coding and noncoding genes and their protein products; hence their inactivation (via DNA methylation), mutation, or misexpression often results in hybrid sterility in one or both sexes. Since gene expression shapes the phenotypes, understanding transcriptomic and proteomic differences between hybrids and their parents would help unravel the biological difference that result in the HMS. Using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), transcriptomic and proteomics, several studies compared the level of gene expression in male sterile hybrid cattle-yak to the fertile parental species. The results showed that the expression of many of the genes involved in the process of spermatogenesis such as cell proliferation and differentiation, germ cell development, and meiosis (pairing and synapsis of homologs, DSB repair, homologous recombination, and crossover formation) is significantly low in the hybrid compared to either parent. The low expression of several of the genes was attributed to DNA methylation of their gene promoter regions, hence affects their mRNA transcription and expression. DNA methylation affects gene expression by directly interfering with the binding of transcription factors to promoters, direct binding of specific transcriptional repressors, or altering chromatin structure [80, 81]. The transcriptomic and proteomic analysis also revealed that most of the differentially expressed genes, proteins, and noncoding RNAs that play key roles in spermatogenesis were downregulated (in the hybrids compared to the parents). For instance, Xu et al. [78] reported that the downregulation miRNAs such as of bta-miR-135a, bta-miR-378, and bta-miR-184 lead to the reduction in SSCs population and affect the process of in spermatogenesis, whereas the downregulation of bta-miR-34b/c and bta-miR-449 could repress the expression of genes (*NANOS3*, *SYCP3*, and *STRA8*) involved in mitosis-to-meiosis transition failure and spermatogenic arrest in the cattle-yak. Genes involved in the accumulation of undifferentiated spermatogonia and severe cell death (*STRA8* and *NLRP14*) were also upregulated in the hybrid. This could be the reason for the presence of few germ cells composed primarily of spermatogonia and Sertoli cells in the seminiferous tubules of bovine hybrids, whereas the parental lines contained abundant germ cells at all stages from the histological data [8, 16, 39, 75, 82]. This reveals that the germ cells did not advance further in the meiotic process. In mammals, deficiency, knock-out, or mutations in genes that regulate these processes leads to arrest in meiosis and the elimination of these defective germ cells via apoptosis [83, 84]. This is the reason for the significantly elevated levels of apoptotic cells (36.197 ± 2.95 vs. yak, 6.907 ± 1.72; $p < 0.001$) found in the cattle-yak testis using TUNEL labeling [39, 40]. Eukaryotic organisms have surveillance mechanisms that monitor the integrity of the genome. The intricate processes involved in meiosis are prone to errors; thus most organisms have evolved surveillance systems

(meiotic checkpoints) to ensure that these processes are devoid of errors. Fundamental aspects of the checkpoint machinery involve sensors to detect errors/damage, signal transducers to transmit the signal, and the eliciting of effector proteins to trigger a suitable biological response. Thus, meiotic checkpoints prevent the generation of defective gametes by either repairing abnormal meiocytes or causing them to self-destruct via apoptosis to eliminate them [85]. It is reported that DNA damage, chromosome asynapsis, unrepaired meiotic DSBs and lack of XY body induce cell-cycle arrest, and the pachytene checkpoint activated so that repair proteins will fix the damage, or the defective cells eliminated via apoptosis pathway [86–88]. At metaphase too, lack of chiasmata, dissociation of homologous autosomes, and XY chromosomes [89, 90] triggers the spindle checkpoint, and the spermatocytes are eventually eliminated by apoptosis. This suggests that the checkpoints monitor the process of meiosis in at least three stages (premeiotic, zygotene-to-pachytene of prophase I, metaphase I), such that a checkpoint is activated to eliminate the defective germ cells at any point they are detected. Thus, the meiotic checkpoints are monitoring systems used by organisms to sense meiotic errors and transduce signals to trigger apoptosis to eliminate the defective to prevent the production of defective gametes, hence play a crucial role in the process of hybrid sterility. The lack of pachytene checkpoint in plants is the reason for the high incidence of unreduced gametes or polyploids among plants compared to animals. Our data (Figure 5; Unpublished) using RT-PCR and 1% agarose gel electrophoresis reaction products revealed that *HOXA4* and *HST1* (a mid-pachynema makers) and *CDC25C* (a late-pachynema maker) expression was detected in cattle and yak testes, but not in the cattle-yak testis, indicating that meiotic arrest before or at mid-pachytene stage. To confirm that the germ cells are eliminated via apoptosis, transcripts of pro-apoptotic genes (*BAX*, *CAS-3*, *6 &7*, *P53*, *TNF-A*, *TRAIL*, and *BMP8B*) were found to upregulated, whereas anti-apoptosis (*BCL-2* and *SURVIVIN*) were downregulated [91]. This suggests that either a process activating apoptosis is potentiated or a process inhibiting apoptosis is compromised in the meiocytes. For instance, the failure of MSCI leads to the falling apart of the sex chromosomes and elimination of the late-pachytene cells by activating the apoptosis cell death pathway [92, 93]. However, MSCI in the cattle-yak has not been studied yet. Taken together, the molecular and histological data suggest that there is dysregulation of important genes that play various roles in spermatogenesis, and their aberrant expression leads to arrest of meiosis and these defective germ cells are subsequently eliminated via apoptosis resulting in lack of postmeiotic germ cells in the tubules of the hybrids. The data reviewed in this work also reveals the polygenic nature of HMS in bovine hybrids involving misexpression of genes with both big and small influence [21, 94].

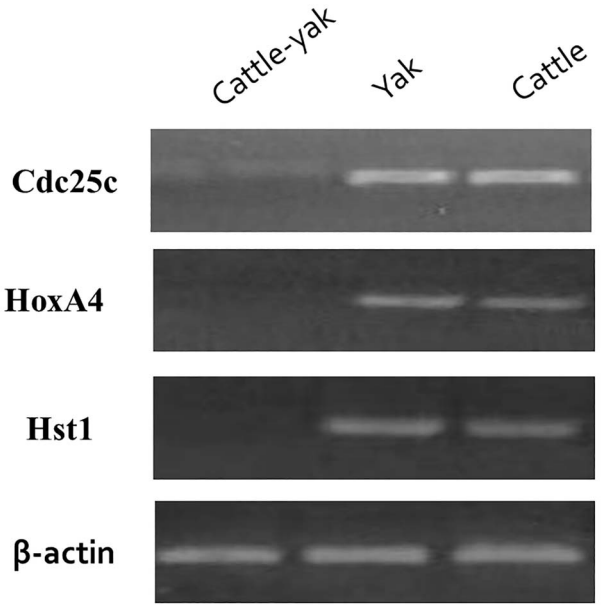

Figure 5. Expression of meiotic arrest marker genes. Agarose gels of *HoxA4* and Hst1 (mid-pachynema markers) and *Cdc25c* (late-pachynema marker) in cattle, yak, and cattle-yak testes. Author's own data.

Conclusion and future perspectives

A major knowledge gap is to determine the root cause of HMS in the cattle-yak. This calls for a comprehensive, multifaceted and targeted approach to address this mystery, which has eluded researchers over the years. Since Peters [17] showed a high degree of asynapsis in cattle/bison hybrids, there is the need to determine if same is true in the cattle-yak and determine the reason for the asynapsis. This will reveal whether the asynapsis is due to aberrations in marking and distribution of recombination hotspots, failure to form DSBs or recognize homologs. We therefore suggest the use of ChIP-Seq single-stranded DNA sequencing on adult testes to determine the distribution of PRDM9, H3K4me3, and H3K36me3 methyltransferases in the hybrid spermatogonia and early spermatocytes to establish if the hotspots are formed normally, and if the individual hotspots in these hybrids are symmetric or asymmetric. If asymmetric binding of PRDM9 alleles to the paternal/maternal genome is found to be the cause, then re-engineering *PRDM9* by replacing the zinc fingers of this gene in bovine hybrids with a human equivalent will be sufficient to restore the symmetrical hotspot formation, hence restore fertility in the male cattle-yak as Davies et al. [61] reported in mice. Also, *SPO11* sequencing is needed to determine whether DSBs are formed normally in the paternal, maternal, and hybrid alongside antibody staining to look at DSB formation, pairing, synapsis, MSCI, normal sex body, and crossover formation in the offspring of fertile female hybrids. This would reveal whether there are any particular regions of the chromosomes that are more or less able to pair, synapse, and undergo recombination. We also suggest studies on targeted single-cell RNA sequencing to identify which genes are dysregulated in which cell type to understand the stage the defects in spermatogenesis possibly started in the cattle-yak. Repeated backcrossing of fertile female hybrids to either of the parent species over several generations while selecting for traits like survivability and sustained heterosis may result in establish a fertile male hybrid with intact heterosis. Addressing this problem will enable farmers and yak breeders to take advantage of the hybrid vigor exhibited by the male cattle-yak hybrid and improve the domestic yak to enhance its productivity.

References

1. Menéndez-Buxadera A, Palacios-Espinosa A, Espinosa-Villavicencio JL, Guerra-Iglesias D. Genotype environment interactions for milk production traits in Holstein and crossbred Holstein-Zebu cattle populations estimated by a character state multibreed model. *Livest Sci* 2016; **191**:108–116.
2. Zhang RC. Interspecies hybridization between yak, Bos taurus and Bos indicus and reproduction of the hybrids. *Recent Advances in Yak*

Reproduction. International Veterinary Information Services, Ithaca, New York. www.ivis.org. Accessed 8 August 2019.
3. Fedyk S, Sysa P. Chromosomes of European bison, domestic cattle and their hybrids. *Acta Theriol* 1971; **16**:26–34.
4. Wurster DH, Benirschke K. Chromosome studies in the superfamily Bovidea. *Chromosoma* 1968; **25**:152–171.
5. Takase H, Tumennasan K, Hiratsuka K, Chandley AC, Hotta Y. Fertility investigation in f1 hybrid and backcross progeny of cattle (Bos taurus) and yak (B. grunniens) in Mongolia: ii. little variation in gene products studied in male-sterile and fertile animals. *Niigata J Health Welfare* 2002; **2**:42–52.
6. Wiener G, Han JL, Long RJ. *The Yak*, 2nd ed. Bangkok, Thailand: Regional Office for Asia and the Pacific Food and Agriculture Organization of the United Nations; 2003: 5–190.
7. Palopoli MF. Wu CI. Genetics of hybrid male sterility between drosophila sibling species: a complex web of epistasis is revealed in interspecific studies. *Genetics* 1994; **138**:329–341.
8. Fedyk S, Krasińska M. Spermatogenesis in backcross generations of European bison and domestic cattle hybrids. *Acta Theriol* 1980; **25**:201–212.
9. Zyczynski A, Kloch M, Olech W. Analysis of spermatogenesis in young male hybrids bred from domestic cattle (Bos taurus) and European bison (Bison bonasus). *Turk J Vet Anim Sci* 2019; **43**:433–440.
10. Coyne JA, Orr HA. *Speciation*. Sunderland (MA): Sinauer Associates; 2004.
11. Maheshwari S, Barbash DA. The genetics of hybrid incompatibilities. *Annu Rev Genet* 2011; **45**:331–355.
12. Haldane JBS. Sex ratio and unisexual sterility in hybrid animals. *J Genet* 1922; **12**:101–109.
13. Laurie CC. The weaker sex is heterogametic: 75 years of Haldane's rule. *Genetics* 1997; **147**:937.
14. Dobzhansky T. *Genetics and the Origin of Species*. New York: Columbia University Press; 1937.
15. Muller. In: Huxley J (ed.), Bearing of the Drosophila work on systematics. The new systematics, vol. **9**. Oxford: Clarendon Press; 1940: 185–268.
16. Fedyk S, Krasińska M. Studies on the spermatogenesis in European bison and domestic cattle hybrids. *Acta Theriol* 1971; **16**:449–464.
17. Peters HF. Experimental hybridization of domestic cattle and American bison. *Proc V Int Congr Anim Reprod Trento VII* 1964; **326**:326–332.
18. Coyne JA. Genetics and speciation. *Nature* 1992; **355**:511–515.
19. Coyne JA, Orr HA. Patterns of speciation in drosophila. *Evolution* 1989; **43**:362–381.
20. Masly JP, Presgraves DC. High-resolution genome-wide dissection of the two rules of speciation in drosophila. *PLoS Biol* 2007; **5**:e243.
21. Turelli M, Orr HA. The dominance theory of Haldane's rule. *Genetics* 1995; **140**:389–402.
22. Wu CI, Davis AW. Evolution of postmating reproductive isolation: the composite nature of Haldane's rule and its genetic bases. *Am Nat* 1993; **142**:187–212.
23. Lifschytz E, Lindsley DL. The role of X-chromosome inactivation during spermatogenesis. *Proc Natl Acad Sci* 1972; **69**:182–186.
24. Royo H, Polikiewicz G, Mahadevaiah SK, Prosser H, Mitchell M, Bradley A, de Rooij DG, Burgoyne PS, Turner JMA. Evidence that meiotic sex chromosome inactivation is essential for male fertility. *Curr Biol* 2010; **20**:2117–2123.
25. Turner JMA, Mahadevaiah SK, Ellis PJI, Mitchell MJ, Burgoyne PS. Pachytene asynapsis drives meiotic sex chromosome inactivation and leads to substantial postmeiotic repression in spermatids. *Dev Cell* 2006; **10**:521–529.
26. Campbell P, Good JM, Nachman MW. Meiotic sex chromosome inactivation is disrupted in sterile hybrid male house mice. *Genetics* 2013; **193**:819–828.
27. Good JM, Giger T, Dean MD, Nachman MW. Widespread overexpression of the X chromosome in sterile F1 hybrid mice. *PLoS Genet* 2010; **6**:e1001148.
28. Bikchurina TI, Tishakova KV, Kizilova EA, Romanenko SA, Serdyukova NA, Torgasheva AA, Borodin PM. Chromosome synapsis and recombination in male-sterile and female-fertile interspecies hybrids of the dwarf hamsters (Phodopus, cricetidae). *Gene* 2018; **9**:227.
29. Ishishita S, Tsuboi K, Ohishi N, Tsuchiya K, Matsuda Y. Abnormal pairing of X and Y sex chromosomes during meiosis I in interspecific hybrids of Phodopus campbelli and P. sungorus. *Sci Rep* 2015; **5**:9435.
30. Dumont BL. Meiotic consequences of genetic divergence across the murine pseudoautosomal region. *Genetics* 2017; **205**:1089–1100.
31. Hale DW, Washburn LL, Eicher EM. Meiotic abnormalities in hybrid mice of the C57BL/6J× Mus spretus cross suggest a cytogenetic basis for Haldane's rule of hybrid sterility. *Cytogenet Genome Res* 1993; **63**:221–234.
32. Otto SP, Pannell JR, Peichel CL, Ashman TL, Charlesworth D, Chippindale AK, Delph LF, Guerrero RF, Scarpino SV, McAllister BF. About PAR: the distinct evolutionary dynamics of the pseudoautosomal region. *Trends Genet* 2011; **27**:358–367.
33. Raudsepp T, Chowdhary BP. The eutherian pseudoautosomal region. *Cytogenet Genome Res* 2016; **147**:81–94.
34. Zhang Q, Li J, Li Q, Li X, Liu Z, Song D, Xie Z. Cloning and characterization of the gene encoding the bovine boule protein. *Mol Genet Genomics* 2009; **281**:67–75.
35. Basrur PK. Hybrid sterility. In: Benirschke K (ed.), *Comparative Mammalian Cytogenetics*. New York: Springer Verlag; 1969: 107–131.
36. Cai X, Yu S, Mipam T, Yang F, Zhao W, Liu W, Cao SZ, Shen L, Zhao F, Sun L, Xu C, Wu S. Comparative analysis of testis transcriptomes associated with male infertility in cattle–yak. *Theriogenol* 2018; **88**:28–42.
37. Liu Z, Li Q, Pan Z, Qu X, Zhang C, Xie Z. Comparative analysis on mRNA expression level and methylation status of DAZL gene between cattle–yak s and their parents. *Anim Reprod Sci* 2011; **126**:258–264.
38. Wu S, Mipam T, Xu C, Zhao W, Shah MA, Yi C, Luo H, Cai X, Zhong J. Testis transcriptome profiling identified genes involved in spermatogenic arrest of cattle-yak. *PLoS One* 2020; **15**:e0229503.
39. Yan P, Xiang L, Guo X, Bao PJ, Jin S, Wu XY. The low expression of Dmrt7 is associated with spermatogenic arrest in cattle-yak. *Mol Biol Rep* 2014; **41**:7255–7263.
40. Lou YN, Liu WJ, Wang CL, Huang L, Jin SY, Lin YQ, Zheng YC. Histological evaluation and PRDM9 expression level in the testis of sterile male cattle–yaks. *Livest Sci* 2014; **160**:208–213.
41. Tripathi UK, Chhillar S, Kumaresan A, Aslam MM, Rajak SK, Nayak S, Manimaran A, Mohanty TK, Yadav S. Morphometric evaluation of seminiferous tubule and proportionate numerical analysis of Sertoli and spermatogenic cells indicate differences between crossbred and purebred bulls. *Veterinary World* 2015; **8**:645.
42. Tumennasan K, Tuya T, Hotta Y, Takase H, Speed RM, Chandley AC. Fertility investigations in the F1 hybrid and backcross progeny of cattle (Bos taurus) and yak (B. grunniens) in Mongolia. *Cytogenet Genome Res* 1997; **78**:69–73.
43. Hale DW. Mammalian spermatogenesis. *Advances in genome biology* 1996; **4**:249–304.
44. Chen WY. Study on chromosome of yak, Holstein, and its hybrid offspring. *China Yak* 1990; **1**:23–29.
45. Guo A. Comparative research on the chromosome in the yak, yellow cattle and its hybrid. *J Genet* 1983; **10**:137–143.
46. Gupta N, Yadav BR, Gupta SC, Kumar P, Sahai R. Chromosomes of domestic yak (Bos grunniens). *Indian J Anim Sci* 1996; **66**: 742–745.
47. Pathak S, Kieffer NM. Sterility in hybrid cattle. II. Distribution of argentophilic structures during male meiosis. In: Pinheiro LEL (ed.), *Proceedings of Advanced Topics in Animal Reproduction Jaboticabal*. Brasil: UNESP; 1980: in press.
48. Luo XL. Study on male cattle's sterility by investigating the historical characteristics of anterior pituitaries and convoluted seminiferous tubules. *J Southwest Coll Nationalities* 1990; **19**:4–13.
49. Liu H, Wang L, Zheng Y. Quantitative histological study of the distal cells of yak, yellow cattle and yak pituitary. *Chinese Yak* 1994; **1**:34–36.

50. Luo X, Wu K, Yang R. The physiological mechanism of normal sexual behaviour of male yak from the diurnal changes of LH, T, P4, 17B-E2 in blood. *Herbivore* 1996; **2**:41–43 48.
51. Cai L, Wiener G. *The yak*. Bangkok, Thailand: Published by the Regional Office for Asia and the Pacific of the Food and Agriculture Organisation of the United Nations; 1995: 1–105.
52. Eddy EM, O'Brien DA. Gene expression during mammalian meiosis. In: *Current Topics in Developmental Biology*, vol. **37**. San Diego, CA, USA: Academic Press; 1997: 141–200.
53. Yu Z, Guo R, Ge Y, Ma J, Guan J, Li S, Sun X, Xue S, Han D. Gene expression profiles in different stages of mouse spermatogenic cells during spermatogenesis. *Biol Reprod* 2003; **69**:37–47.
54. Schübeler D. Function and information content of DNA methylation. *Nature* 2015; **517**:321–326.
55. Baudat F, Buard J, Grey C, Fledel-Alon A, Ober C, Przeworski M, Coop G, de Massy B. PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. *Science* 2010; **327**:836–840.
56. Mihola O, Trachtulec Z, Vlcek C, Schimenti JC, Forejt J. A mouse speciation gene encodes a meiotic histone H3 methyltransferase. *Science* 2009; **323**:373–375.
57. Paigen K, Petkov PM. PRDM9 and its role in genetic recombination. *Trends Genet* 2018; **34**:291e300.
58. Hayashi K, Yoshida K, Matsui Y. A histone H3 methyltransferase controls epigenetic events required for meiotic prophase. *Nature* 2005; **438**:374.
59. Li YC, Wang GW, Xu SR, Zhang XN, Yang QE. The expression of histone methyltransferases and distribution of selected histone methylations in testes of yak and cattle-yak hybrid. *Theriogenol* 2020; **144**:164–173.
60. Sun F, Fujiwara Y, Reinholdt LG, Hu J, Saxl RL, Baker CL, Petkov PM, Paigen K, Handel MA. Nuclear localization of PRDM9 and its role in meiotic chromatin modifications and homologous synapsis. *Chromosoma* 2015; **124**:397–415.
61. Davies B, Hatton E, Altemose N, Hussin JG, Pratto F, Zhang G, Hinch AG, Moralli D, Biggs D, Diaz R, Preece C. Re-engineering the zinc fingers of PRDM9 reverses hybrid sterility in mice. *Nature* 2016; **530**: 171–176.
62. Ahlawat S, De S, Sharma P, Sharma R, Arora R, Kataria RS, Datta TK, Singh RK. Evolutionary dynamics of meiotic recombination hotspots regulator PRDM9 in bovids. *Mol Genet Genomics* 2017; **292**: 117e31.
63. Sandor C, Li WB, Coppieters W, Druet T, Charlier C, Georges M. Genetic variants in REC8, RNF212, and PRDM9 influence male recombination in cattle. *PLoS Genet* 2012; **8**.
64. Barski A, Cuddapah S, Cui KR, Roh TY, Schones DE, Wang ZB, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. *Cell* 2007; **129**(4):823–837.
65. Cao R, Wang LJ, Wang HB, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. Role of histone H3 lysine 27 methylation in polycomb-group silencing. *Science* 2002; **298**:1039e43.
66. Metzger E, Wissmann M, Yin N, Muller JM, Schneider R, Peters AHFM, Günther T, Buettner R, Schüle R. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* 2005; **437**:436e9.
67. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, Reinberg D, Jenuwein T. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev* 2004; **18**:1251e62.
68. Griswold MD. The central role of Sertoli cells in spermatogenesis. *Semin Cell Dev Biol* 1998; **9**:411e6.
69. Li MG, Liu ZS, Pan ZX, Hua L, Xie Z, Li QF. The mRNA expression and methylation status in imprinting control region of H19 gene between cattle–yak and their parents. *J Integr Agric* 2012; **11**:1691–1699.
70. Leighton PA, Saam JR, Ingram RS, Stewart CL, Tilghman SM. An enhancer deletion affects both H19 and Igf2 expression. *Genes Dev* 1995; **9**:2079–2089.
71. Abane R, Mezger V. Roles of heat shock factors in gametogenesis and development. *FEBS J* 2010; **277**:4150–4172.
72. Liu P, Yu S, Cui Y, He J, Zhang Q, Liu J. Regulation by HSP70/90 in the different tissues and testis development of male cattle (cattle–yak and yak). *bioRxiv* 2018; 393371. doi: 10.1101/393371.
73. Liu P, Yu S, Cui Y, He J, Zhang Q, Sun J. Regulation by Hsp27/P53 in testis development and sperm apoptosis of male cattle (cattle-yak and yak). *J Cell Physiol* 2019; **234**:650–660.
74. Hu Y, Fu A, Miao Z, Zhang X, Wang T, Kang A, Shan J, Zhu D, Li W. Fluorescent ligand fishing combination with in-situ imaging and characterizing to screen Hsp 90 inhibitors from Curcuma longa L. based on InP/ZnS quantum dots embedded mesoporous nanoparticles. *Talanta* 2018; **178**:258–267.
75. Xu Y, Diao Y, Qi S, Pan X, Wang Q, Xin Y, Cao X, Ruan J, Zhao Z, Luo L, Liu C, Yin Z. Phosphorylated Hsp27 activates ATM-dependent p53 signalling and mediates the resistance of MCF-7 cells to doxorubicin-induced apoptosis. *Cell Signal* 2013; **25**:1176–1185.
76. Mukherjee A, Koli S, Reddy KVR. Regulatory non-coding transcripts in spermatogenesis: shedding light on 'dark matter'. *Andrology* 2014; **2**:360–369.
77. Robles V, Herraez P, Labbé C, Cabrita E, Pšenička M, Valcarce DG, Riesco MF. Molecular basis of spermatogenesis and sperm quality. *Gen Comp Endocrinol* 2017; **245**:5–9.
78. Xu C, Wu S, Zhao W, Mipam T, Liu J, Liu W, Yi C, Shah MA, Yu S, Cai X. Differentially expressed microRNAs between cattle–yak and yak testis. *Sci Rep* 2018; **8**:592.
79. Ponting CP, Oliver PL, Reik W. Evolution and functions of long non-coding RNAs. *Cell* 2009; **136**:629–641.
80. Hou Y, Yuan J, Zhou X, Fu X, Cheng H, Zhou R. DNA demethylation and USF regulate the meiosis-specific expression of the mouse Miwi. *PLoS Genet* 2012; **8**:e1002716.
81. Huang YZ, Zhang LZ, Lai XS, Li MX, Sun YJ, Li CJ, Lan X-Y, Lei C-Z, Zhang C-L, Zhao X, Chen H. Transcription factor ZBED6 mediates IGF2 gene expression by regulating promoter activity and DNA methylation in myoblasts. *Sci Rep* 2014; **4**:4570.
82. Sun L, Mipam TD, Zhao F, Liu W, Zhao W, Wu S, Xu C, Yu S, Cai X. Comparative testis proteome of cattle–yak from different developmental stages. *Animal* 2017; **11**:101–111.
83. Hunt PA, Hassold TJ. Sex matters in meiosis. *Science* 2002; **296**:2181–2183.
84. Aitken RJ, Findlay JK, Hutt KJ, Kerr JB. Apoptosis in the germ line. *Reproduction* 2011; **141**:139.
85. Jaramillo-Lambert A, Harigaya Y, Vitt J, Villeneuve A, Engebrecht J. Meiotic errors activate checkpoints that improve gamete quality without triggering apoptosis in male germ cells. *Curr Biol* 2010; **20**:2078–2089.
86. De Vries FA, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, Liu J-G, van Zeeland AA, Heyting C, Pastink A. Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. *Genes Dev* 2005; **19**:1376–1389.
87. Pittman DL, Cobb J, Schimenti KJ, Wilson LA, Cooper DM, Brignull E, Handel MA, Schimenti JC. Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. *Mol Cell* 1998; **1**:697–705.
88. Shi B, Xue J, Yin H, Guo R, Luo M, Ye L, Shi Q, Huang X, Liu M, Sha J, Wang PJ. Dual functions for the ssDNA-binding protein RPA in meiotic recombination. *PLoS Genet* 2019; **15**:e1007952.
89. Mark M, Jacobs H, Oulad-Abdelghani M, Dennefeld C, Féret B, Vernet N, Ghyselinck NB. STRA8-deficient spermatocytes initiate, but fail to complete, meiosis and undergo premature chromosome condensation. *J Cell Sci* 2008; **121**:3233–3242.
90. Oka A, Mita A, Takada Y, Koseki H, Shiroishi T. Reproductive isolation in hybrid mice due to spermatogenesis defects at three meiotic stages. *Genetics* 2010; **186**:339–351.
91. Zeng X, Chai Z, Wang Y, Ma Z, Yang Q, Song Q, Zhong JC. Comparative transcriptome analysis of spermatogenesis arrest in cattle–yak. *Scientia Sinica Vitae* 2014; **44**:584–601.
92. Fernandez-Capetillo O, Celeste A, Nussenzweig A. Focusing on foci: H2AX and the recruitment of DNA-damage response factors. *Cell Cycle* 2003; **2**:425–426.

93. Ichijima Y, Ichijima M, Lou Z, Nussenzweig A, Camerini-Otero RD, Chen J Namekawa SH. MDC1 directs chromosome-wide silencing of the sex chromosomes in male germ cells. *Genes Dev* 2011; **25**:959–971.
94. Maside XR, Naveira HF. A polygenic basis of hybrid sterility may give rise to spurious localizations of major sterility factors. *Heredity* 1996; **77**:488–492.
95. He S, Ma Y, Wang J, Li Q, Yang X, Tang S, Li H. Milk fat chemical composition of yak breeds in China. *J Food Compos Anal* 2011; **24**:223–230.
96. Ding FH, Wei YP, Xu JT, Tong ZB, Ma ZJ, Chen SM, You-La CR, Lou XL. The comparisons of the performance of yak, Pian Niu and yellow cattle, and the determination of flavour in the muscle. *J Qinghai Univ* 2008; **26**:7–10.
97. Brasch S. *10 Farm Animal Hybrids You Didn't Know Existed*. Modern Farmer, 2014. https://bit.ly/2YY1RNc. Accessed 13 March, 2020.
98. Deakin A, Muir GW, Smith AG. Hybridization of domestic cattle, bison and yak. Report on the Warinwright experiment. *Publ Dep Agric Can* 1935; **479**:30.
99. Logan NVS, Svlvestere PE. *Hybridization of Domestic Beef Cattle and Buffalo*. Can Dept Agr 1950. Progress Report, Ottawa.
100. Nichols J. *Broadcasting the Beefalo Blog: Buffalo More Than Outstanding Beef*. Beefalo Northwest Association, 2018. https://bit.ly/2Mospk9. Accessed 24 June, 2020.
101. Łozicki A, Olech W, Dymnicka M, Florowski T, Adamczak L, Arkuszewska E, Niemiec T. Nutritive value and meat quality of domestic cattle (Bos taurus), zubron (Bos taurus × Bison bonasus) and European bison (Bison bonasus) meat. *Agric Food Sci* 2017; **26**:118–128.
102. Madsen DB, Chen F, Gao X. *Late Quaternary Climate Change and Human Adaptation in Arid China*, vol. **207**. Elsevier; 2007.
103. Anonymous. Dzo facts. SoftSchools.com. 2019k. https://bit.ly/2HgJ4Sq. Accessed 15 July 2020.
104. Zhang Q, Li Q, Li J, Li X, Liu Z, Song D, Xie Z. b-DAZL: a novel gene in bovine spermatogenesis. *Prog Nat Sci* 2008; **18**:1209–1218.
105. Yao W, Li Y, Li B, Luo H, Xu H, Pan Z. Epigenetic regulation of bovine spermatogenic cell-specific gene boule. *PLoS One* 2015; **10**: e0128250.
106. Han Y, Fu Y. An investigation on the relationship between the azoospermia-like (DAZL) gene mRNA expression and the infertility in male cattle–yak. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 2016; **22**:287–289.
107. Luo H, Jia C, Qu XG, Zhao XB, Zhong JC, Xie Z, Li QF. Cloning and testicular expression level of b-Sycp2 in cattle, yak and cattle–yak. *Sci Agri Sin* 2013; **2**:367–375.
108. Qu XG, Li QF, Liu ZS, Dong LY, Li XF, Hao CL, Xie Z. The study on the expression level of SYCP3 mRNA in yak and cattle–yak testis. *Chinese J Anim Vet Sci* 2008; **8**.
109. Wang S, Pan Z, Zhang Q, Xie Z, Liu H, Li Q. Differential mRNA expression and promoter methylation status of SYCP3 gene in testes of yaks and cattle-yaks. *Reprod Domest Anim* 2012; **47**:455–462.
110. Li B, Luo H, Qu XG, Pan ZX, Xie Z, Liu HL, Qifa L. The expression pattern of synaptonemal complex protein b-FKBP6 gene in yak and cattle–yak hybrid. *J Nanjing Agri Univ* 2013; 129–132.
111. Li B, Luo H, Weng Q, Wang S, Pan Z, Xie Z, Wu W, Liu H, Li Q. Differential DNA methylation of the meiosis-specific gene FKBP 6 in testes of yak and cattle-yak hybrids. *Reprod Domest Anim* 2016; **51**:1030–1038.
112. Dong L, Li Q, Qu X, Li Y, Li X, Hu HT, Xie Z. Expression levels of Cdc2 and Cdc25A mRNA in cattle, yak, and cattle–yak testis. *Yi chuan= Hereditas* 2009; **31**:495–499.
113. Yang Z, Hua L, Bo-Jiang LI, Chao JIA, Zhuang XIE, Xing-bo Z, Jin-Cheng Z, Qi-Fa L. mRNA expression level and promoter methylation of DDX4 gene in testes of yak and cattle–yak. *Sci Agr Sin* 2013; **46**:630–638.
114. Jin S, Guo X, Bao PJ, Niang CN. Sequence analysis and study on the expression level of Dmrt7 gene in yak and cattle-yak testis. *Sci Agr Sin* 2013; **46**:1036–1043.
115. Li X, Li Q, Zhao X, Xu H, Gu Y, Zhu X, Zhuang X, Hong-Lin L. Sequence analysis and study on the expression level of Dmc1 mRNA in yak and cattle–yak testis. *Sci Agr Sin* 2010; **43**:3221–3229.
116. Li B, Wu W, Luo H, Liu Z, Liu H, Li Q, Pan Z. Molecular characterization and epigenetic regulation of Mei1 in cattle and cattle–yak. *Gene* 2015; **573**:50–56.
117. Pan Z, Liu Z, Li Y, Yu S, Li M, Xie Z, Li Q-F. Difference of SNRPN methylation status and its mRNA expression in testes between cattle–yak s and their parents. *Sci Agr Sin* 2010; **43**:4709–4716.
118. Gu Y, Li Q, Pan Z, Li M, Luo H, Xie Z. Molecular cloning, gene expression and methylation status analysis of PIWIL1 in cattle-yaks and the parental generation. *Anim Reprod Sci* 2013; **140**:131–137.
119. Liu Z, Li Q, Zhang Q, Qu X, Dong L, Yang-zom C. Analysis of IGF2 mRNA expression and its methylation status between cattle yaks and their parents. *Prog Nat Sci* 2009; **19**:1063–1069.
120. Wang PF, Huang L, Tang HH, Jin SY, Zheng YC. Expression of Prm1 and Prm2 gene in the testes of yaks and sterile cattle–yak s. *Hubei Agri Sci* 2016; **20**:55.
121. Huang L, Jin SY, Xu YO, Li YP, Lin YQ, Zheng YC. Quantitation of alternative splicing variants of lactate dehydrogenase C gene in testes of adult yak, sexually immature yak calf and sterile male hybrid of yak. *Can J Anim Sci* 2012; **92**:291–296.
122. Zhang GW, Wu Y, Luo Z, Guan J, Wang L, Luo X, Zuo F. Comparison of Y-chromosome-linked TSPY, TSPY2, and PRAMEY genes in Taurus cattle, yaks, and interspecific hybrid bulls. *J Dairy Sci* 2019; **102**:6263–6275.
123. Yu S, Cai X, Sun L, Zuo Z, Mipam T, Cao S, Shen L, Ren Z, Chen X, Yang F, Deng J, Ma X et al. Comparative iTRAQ proteomics revealed proteins associated with the spermatogenic arrest of cattle–yak. *J Proteomics* 2016; **142**:102–113.
124. Yang F, Mipam T, Sun L, Yu S, Cai X. Comparative testis proteome dataset between cattle–yak and yak. *Data Brief* 2016; **8**:420–425.
125. Guo AY, Xiong XR, Wang Y. Identification and analysis of Long non-coding RNA associated with cattle–yak male infertility. *Acta Veterinaria Et Zootechnical Sinica* 2019; **50**:551–561.
126. Wang H, Zhong J, Chai Z, Zhu J, Xin J. Comparative expression profile of micro RNAs and piRNA s in three ruminant species testes using next-generation sequencing. *Reprod Domest Anim* 2018; **53**:963–970.