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Male Reproductive Physiology (28-Nov-2000)

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Summary
A comprehensive description of reproductive physiology of yak bulls has been compiled. Spermatogenesis in the yak is similar to that in other mammals. Distinctive characteristics of spermatogenesis in the yak and fine structures of yak spermatozoa are described.

Introduction
Yaks, a unique breed of cattle, are adapted to the alpine grassland on the Qinghai-Tibet Plateau at an altitude exceeding 3000 m. Yaks are raised under extensive conditions, continuously grazing pastures of limited nutritional value. The majority of the breeding is natural service with artificial insemination limited to a few specific regions.

Spermatogenesis in the Yak Bull
Spermatogenesis in the yak, a lengthy but precisely controlled chronological process in which spermatozoa are produced in the seminiferous tubules, is similar to that in other species. Spermatogenesis is divided into three major divisions: spermatocytogenesis, meiosis and spermiogenesis [1]. Stem cell spermatogonia divide by mitosis to maintain their population and to produce primary spermatocytes; the latter cells undergo meiosis to produce haploid spermatids that differentiate into spermatozoa.

Spermatogenetic Cycle and the Seminiferous Epithelium Cycle
The spermatogenetic cycle includes all the events that occur between two appearances of the same developmental stage (steps) [2]. These stages are defined by the morphologic appearance of the germ cells in PAS-stained sections. Xia et al., [14] reported that the spermatogenetic cycle and the seminiferous epithelium phase of yak bulls are very similar to that in cattle, reported by Berndtson et al., [4]. It appears that the VII phase and the XI phase are very long, yet the IX, X, and XII phase are rather shorter [3,4].

Qualitative and Quantitative Aspects of Spermatogenesis
Oravant et al., [5] and Ekstedt et al., [6] suggested that spermatogonia in bulls undergo five or six mitotic divisions, during which their structure and nuclear size gradually change. Hochereau de Reviers [7] found six mitotic divisions and thought that there are two generations of B spermatogonia. Similarly, there are two types of B spermatogonia in yaks; A spermatogonia divide to form B_1 spermatogonia that subsequently divide to form B_2 spermatogonia. During mitotic division of the A spermatogonia, some become quiescent and others continue to divide. In theory, after six mitotic divisions, there would be 2^6 (= 64) spermatids. However, due to degeneration, cell numbers do not increase by the theoretical value; 26.7% of A spermatogonia degenerate when they differentiate into the intermediate type. One A spermatogonium produces 26.95 round spermatids (42.1% of the theoretical yield) in the Yak [3] and 27.23 round spermatids in the bull.
Spermatogonia undergo mitosis to form spermatocytes. The numbers of primary spermatocytes remain relatively stable at the stage of proleptonema, leptonema, zygotene, and the long pachytene period. There are an average of 30.8 secondary spermatocytes in each seminiferous tubule cross section in yaks, close to double the number of primary spermatocytes ($15.9 \times 2 = 31.8$), indicating that few cells at this stage are lost by degeneration. However, these form 55.4 spermatids, indicating that losses during meiosis are approximately 10% of the theoretical value ($30.8 \times 2 = 61.6$). However, the number of Sertoli cells remains relatively constant throughout the seminiferous epithelium cycle [8,9].
Xia [8] concluded that the numbers of both Sertoli cells and all kinds of germ cells in yak testes are approximately 80% of those in cattle; he attributed this to breed differences and the effects of nutrition. The rate of seminiferous cell production in yaks is similar to that in cattle during spermatogenesis; however, the proliferation rate in yak is about 10% lower than in cattle.

The Quantitative Histology of Testes

Yak testes are anatomically and histologically similar to those of other bovine bulls except that they are smaller (approximately 300 versus 550 - 650 g, respectively). The yak scrotum is small with abundant hair, apparently an adaptation to the cold environment [10].

The weight and volume of the testes are closely related to their ability to produce sperm. Intrinsic factors affecting the weight and volume of testes include diameter of the seminiferous tubule, height of the seminiferous epithelium, the amount of interstitial tissue, and the level of spermatogenesis. Environmental factors, age, and hormones also affect testicular weight and volume. The height of the seminiferous epithelium and volume density of seminiferous tubules and seminiferous epithelium increase with age, but the volume density of the lumen of the seminiferous tubule and interstitial tissue decrease gradually with age. At 24 months of age, the volume density of seminiferous tubule of yak is 0.786 $\mu m^3/\mu m^3$, the volume density of seminiferous epithelium is 0.677 $\mu m^3/\mu m^3$, the height of seminiferous epithelium is 85.66 μm, and the volume percentage of seminiferous tubules is 78.6%, similar to that in mature males of common cattle breeds (79.4%) [11].

Daily sperm production per gram of testicular parenchyma, a measure of the efficiency of spermatogenesis, is useful for comparisons. Yaks have an efficiency of 12.94×10^6/g, similar to other cattle (e.g., 13.00×10^6/g and 12×10^6/g in Charolais and Holstein bulls, respectively). Total daily sperm production in yaks is 4.66×10^9, less than that of *Bos taurus* bulls (e.g., 8.9×10^9 and 7.5×10^9 in Charolais and Holstein bulls, respectively) but greater than Nile Zebu bulls (2.6×10^9). The difference is due to the different index of testes. Furthermore, compared to the Yak, testicular weight is 2.15, 2.01 and 0.65 times higher in Charolais, Holstein and Nile Zebu bulls, respectively. The height of the seminiferous epithelium is 61 and 102μm in Zebu and *Bos taurus* bulls, respectively [11-13].

Sperm Morphology

The yak sperm consists of a head, neck and tail that are very similar to those of other cattle breeds. The tail is composed of a midpiece, principal piece and endpiece.

In yaks, the head of a sperm has a reverse oval shape; the front two-thirds are enclosed by the acrosome, the final one-third lies in the post nuclear cap, and the nucleus is located in the middle. The nucleus appears as a reverse flattened oval, enclosed in the nuclear membrane. The acrosome is a sheath that covers the front two-thirds of the sperm's head. It is composed of outer and inner acrosomal membranes and the acrosomal inclusion (moderately dense and uniform), located within the inner acrosomal membrane. The outer acrosomal membrane protrudes to form an acrosome ridge along the upper aspect of the sperm head. The inner acrosomal membrane coils and projects a sharp tip (containing moderately dense material), similar to the perforatorium of avian and rodent sperm. The inner and outer acrosomal membranes come together to form the equatorial segment (ES); this is usually visible by light microscopy in Giemsa-stained sperm and is even more distinct in abnormal sperm that lack an acrosome. The postacrosomal sheath covers the region of the sperm head below the equator [14].

The plasmalemma (protoplasmic membrane) completely covers the sperm head and tail. The plasmalemma covering the anterior of the sperm head (in apposition with the outer acrosomal membrane) is relatively labile while that below the equator is more stable.

The middle of the lower part of the sperm's head is depressed to form an implantation fossa that accommodates the implantation plate of the sperm tail. The neck consists of nine outer-segment, cylinder-shaped implantation plates. These nine plates are combined closely into the joint lump; the front of the joint lump sticks out and forms a hemi-spherical structure that inserts into the implantation fossa. In the center of the implantation fossa is the near centriole. Although the form and size of the section of the nine implantation plates vary, they become more uniform distally.

The middle piece of the tail is composed of an axial filament that is covered by a mitochondrial sheath. The axial filament consists of 9 thick fibrils, 9 microtubule doublets and 2 center microtubules. The mitochondrial sheath is comprised of a mitochondrial helix covered by a protoplasmic membrane.

The principal piece (sperm tail) is made up by the mid-axial filament and fibrils sheath. The mid-axial filament continues from the mid piece but only the third and the eighth strand fibril elongate distally; many cross ribs are joined from two sides to form the fibrils sheath (in lieu of the mitochondrial sheath of the mid piece). The fibrils sheath becomes slim gradually, and the microtubule doublet varies in the same way.

The telopiece of sperm has lost the fibrils sheath and the thick fiber; the only structure remaining is the mid axial-filament covered by a protoplasmic membrane.

Compared to sperm from *Bos taurus* bulls, the dimensions for yak sperm are 9.50 vs. 8.32 μm, the length of the midpiece is 14.20 vs.14.40 μm ($P<0.05$), and the length of the principal piece is 51.83 vs. 47.5 μm ($P<0.01$) [14].

Sexual Activity

Yak bulls start mounting around 6 months of age; over the next year, this behavior continues and intensifies, including seeking and mounting yak females. Many studies in Yaks suggest that spermatogonia begin to differentiate at 12 months of age and that sperm are present in the epididymis by 18 months of age. However, the LDH-x band, a marker characteristic of

sexual maturity in mammals, is not present until 24 months of age.
Bulls spend winter and early spring alone, joining the herd only during the breeding season. Bulls can detect the scent of an estrus female from a distance of several kilometers. There is considerable fighting among bulls, with the strongest, most dominant bulls getting the majority of the opportunities to mate. Young bulls do not usually win a place in the competition for mates until they are approximately 4 years old (after some fighting experience). Old feeble bulls do not have mating opportunities and leave the herd. The competition for mates, to the extent that it introduces an additional element of natural selection, provides the yak with some advantages in surviving in a harsh environment. Furthermore, by ensuring that old bulls are generally replaced before their daughters have reached breeding age, this competition for dominance may also reduce the degree of inbreeding. However, with the exception of human intervention, there is nothing to prevent bulls from mating their siblings or succeeding their sires in the herd.
Bulls that have won a mating position in the herd usually mate several times a day. During mating, bulls will not attack other bulls, unless strongly provoked.
The interval during which Yak bulls are suitable for mating is breed dependent. The Tibet Mendui yak is suitable for breeding between 3.5 and 8 years (ideally 4.5 to 6.5 years) with few used after 8 years. Gannan Maqu bulls are used from 3.5 to 14 years and Qinghai Haibei bulls from 4 to 10 years. In Sichuan Jiulong, the period is from 4 to 12 years (with best results from 6 to 10 years). According to the studies on semen collection and semen quality evaluation conducted for many years in Datong Yak Farm, Qinghai, Yak bulls reach their reproductive peak at 7 years and gradually decline thereafter, with the best results between 4 and 8 years of age.
A sexually productive life expectancy of not more than 10 years for a yak bull is reinforced by results from an AI stud of 38 yak bulls in Tibet (altitude, 4300 m) where ejaculate volume, concentration and motility of sperm rose steadily from the age of 3 to 9 years and subsequently declined [15].

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