Abstract

Breeding is one of the most important aspects of livestock husbandry. Among the many factors that have a significant impact on maintaining a healthy and fertile herd is to determine the male reproductive potential and selection of the right male for breeding. Analysis of the location and expression of aquaporins (AQPs) in male reproductive organs seems to be helpful in this respect. In spite of many published data on AQPs in male laboratory animals and humans, still little is known on this subject in livestock, including cattle. Therefore, studies were undertaken with the main aims including (I) identification, detailed localization and analysis of changes in the expression of aquaglyceroporins and classical aquaporins in the reproductive tract of bulls and (II) determination of the potential role of these proteins in growth and development, proper course of reproductive processes and semen production.

The study was conducted in three age groups of cattle (*Bos taurus*) of the Polish Holstein-Friesian breed of the Black-and-White variety: (I) calves aged 5 to 6 weeks ($n = 10$), (II) young bulls aged 15 to 25 weeks ($n = 10$) and (III) reproductive bulls aged 2 to 6 years ($n = 11$). Immediately after slaughter, the reproductive organs were removed and representative fragments of the testis, epididymis and vas deferens were dissected. The prepared histological sections were subjected to morphological and morphometric analyses using hematoxylin-eosin, periodic acid-Schiff reagent, and Masson's trichrome. Identification, detailed localization and analysis of the expression of AQPs was conducted using immunohistochemical staining and/or Western blot technique. In order to properly assess the distribution of individual AQPs, Sertoli and Leydig cells were also identified in all studied animals using anti-GATA-4 antibodies.

Based on the obtained results, it was found that typical and characteristic morphological changes occur in the reproductive organs with growth and development of the studied animals. These changes included, among others, an increase in the diameter of the seminiferous tubules, germ cell development and differentiation of the epithelium of the epididymis and vas deferens. The analysis of GATA-4 expression allowed to conclude that in calves and young bulls nearly half of the cells lining the seminiferous epithelium were immature Sertoli cells, while numerous Leydig cells were observed in the intertubular space.

Three aquaglyceroporins (AQP3, AQP7, and AQP9) and five classical aquaporins (AQP0, AQP1, AQP4, AQP5, and AQP6) have been identified in specific cell types and regions of the male reproductive tract in cattle. Among many observations and statements, it is worth mentioning the presence of AQP3 and AQP7 in gonocytes, where these proteins are most likely involved in their proliferation and migration. In reproductive bulls, AQP3 was observed in

spermatogonia and spermatocytes, while AQP7 in all germ cells types and in Sertoli cells. Expression of both proteins in germ cells changed with growth and development of animals. In sexually mature individuals, the presence of AQP1 was also recorded in peritubular myoid cells, where this protein most likely supports the transport of immotile spermatozoa from the lumen of the seminiferous tubules to the subsequent segments of the male reproductive system. In the studied individuals, AQP0, AQP7 and AQP9 were located in Leydig cells. Within the epithelium of rete testis and efferent ducts, AQP0, AQP3, AQP6 and AQP7 were observed. Along the successive segments of the epididymis in individual cell types and their regions, the presence of AQP0, AQP1, AQP3, AQP4, AQP5, AQP6, AQP7 and AQP9 was recorded. Their mutual distribution and differences in expression indicate not only the key role of these proteins in the creation of a unique microenvironment in the epididymal lumen, but also the influence of other factors, including hormones on their regulation. In the cells lining the epithelium of the vas deferens, as many as six AQPs were observed, including AQP3, AQP4, AQP5, AQP6, AQP7 and AQP9, which indicates extensive transport of water and other small molecules also in this section.

As a result of the conducted studies, a total of eight AQPs, i.e. AQP0, AQP1, AQP3, AQP4, AQP5, AQP6, AQP7 and AQP9 were identified in the male reproductive system in cattle. It should be noted that in the present study, for the first time, gonocytes were analyzed for the presence of AQPs and AQP6 was identified in male reproductive organs. Based on the obtained results, the potential role of individual AQPs in the reproductive system of the bull was also formulated. These include participation, among others, in the proliferation and migration of gonocytes, in the differentiation of germ cells, in the transport of spermatozoa from the lumen of the seminiferous tubules and in the formation of the appropriate environment for sperm maturation and storage in the epididymis. The results presented in this paper indicate the important role of AQPs in the proper development and course of reproductive processes in bulls.

Keywords: water channel, morphology, GATA-4, reproduction, testis, rete testis, efferent ducts, epididymis, vas deferens

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