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Anatomy of the Reproductive Tract of the Female and Male Buffaloes

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The normal anatomy of the female and male genital system is important to understand in view of the physiological clinical procedures that need to be undertaken and also to understand the various structures. The female reproductive tract comprises of the tubular genitalia and the generative organs [1-5] whereas the male reproductive tract consists of the testes and the tubular structures and the accessory sex glands [2,3]. Cattle and buffalo appear to be similar in the anatomy of the female and male reproductive tracts yet some differences such as a narrower cervix, smaller ovarian and testicular dimensions and smaller male accessory sex glands in the buffalo render the species slightly different from cattle. In this chapter the authors describe the anatomy of the reproductive tract of the female and male buffalo.

1. Anatomy of the Female Buffalo Reproductive Tract

1.1. Importance of the Anatomy of the Female Reproductive System

Knowledge of female anatomic particularities is important, among other things, for the employment of reproductive techniques such as pregnancy diagnosis, artificial insemination, superovulation, ovum pick up and embryo transfer.
The female genital system includes the ovaries, which produce gametes and hormones; the oviducts, which are extensions of the uterus and provide transport functions for oocytes, sperm and secretions; the uterus, which is the site of embryonic and fetal development; the vagina, which is the channel for the passage of copulation and the fetus, and the external genitalia [1-5]. The internal genitals are held by the broad ligament, comprising the mesovary that supports the ovary, the mesosalpinx which support the oviducts, and the mesometrium, that supports the uterus [3].

According to Brar et al. [6], the broad ligament is the only attachment of the uterus with the body of the animal. In general, the broad ligaments consist of double serosal folds containing in between them muscles, connective tissue, nerves and blood vessels [6]. Brar et al. [6], studying broad ligaments in buffaloes observed that: in 0 day-old calves the fibroblasts and muscle fibers were still to be organized; the adult non-pregnant buffaloes had well-developed musculature in broad ligaments; the pregnant side of the broad ligament had relatively larger muscle fascicles with frequent syncytial appearances of muscle fibers.

1.2. The Bubaline Pelvis

The bubaline pelvis is different from the bovine pelvis [7,8]. The pelvic inlet in buffalo is more circular and oblique compared to elliptical in cows [8]. The two symphysis pubis are fused together completely in the buffalo with a gap of variable length [7]. The ischium bone in buffalo forms an angle of 70 degrees with the horizontal (Fig. 1) compared to 50 degrees in cows [8].

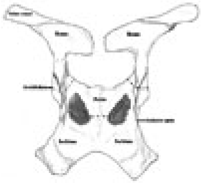

Figure 1. The diagram of bubaline pelvis. (Photo courtesy Prof G.N. Purohit, Department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Sciences, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India). - To view this image in full size go to the IVIS website at www.ivis.org . -

The superior ischiatic spine is high, thin, irregularly convex, sharp and fragile in buffaloes [7]. The tuber ischii is large and "y" shaped in buffalo [8]. The distance between the acetabulum and tuber coxae, and between the tuber ischii and acetabulum is nearly equal (with difference of 4-5 cm) in cattle, whereas in buffaloes the difference of 8-10 cm exists [7].

The 5th sacral vertebra is loosely attached with the 4th sacral vertebra in buffalo whereas in the cow the 4th and 5th sacral vertebra are fused [7]. The body of the first sacral segment is very wide and is more flattened dorsoventrally in the buffalo [7]. Collectively these variations result in significantly greater transverse and sacropubic diameters, pelvic outlet, pelvic inlet, vertical and diagonal pelvic diameters in the buffalo compared to cows [8].

1.3. Buffalo Female External and Internal Genitals

Cattle and buffalo are considered similar species yet some differences in the female external and internal genitals exist. The tubular genitalia of the female buffalo are in general more muscular and rigid and the uterine cornua are more tortuous than those of the cow. The cervix of the buffalo is narrower than that of the cow [9] and the cervix of Egyptian buffaloes is a little shorter than that of the Indian breeds [10]. The ovaries of the buffalo although considered similar to those of cattle, are in general smaller in size. The number of primordial follicles in the buffalo ovaries are comparatively too small [11] compared to those in cattle and the follicle diameters are smaller in the buffalo [12]. The corpus luteum is less projecting on the ovarian surface and more embedded in the ovarian stroma [13]. The oviducts of the buffalo are coarser and more deeply embedded in the broad ligaments than in the cow.

The morphology of the genital system of buffaloes is often compared to that of cows (Fig. 2) and the earliest descriptions [14] mention that the female genitals of the swamp and river buffaloes are similar to those of dairy cows. According to Sane et al. [15], the genital system of Murrah buffalo female is more muscular and hard, and the uterine horns are more tortuous than those of cows. However, the same authors reported that the bovine female genital system is slightly larger and heavier than in the buffalo. These comparisons were performed using the European cattle breeds as a basis, which are larger and heavier than Indian breeds. It is likely that cows and buffaloes with similar body size and weight also have similarity in the biometry of genital systems. Working with Surti buffalo heifers, Danell [11] found a positive correlation between body weight and size and weight of the genital system.

Figure 2. Schematic diagram of the buffalo's (left) and cattle (right) female genital systems and their abattoir derived images. - To view this image in full size go to the IVIS website at www.ivis.org . -

The closed portion of the genital system includes the ovaries, oviducts, uterine horns and the uterine body, while the open portion comprises the vagina and external genitalia. The biometry of the genital system of cattle and buffaloes, as reported by different authors (Table 1 and Table 2), reflect marginal differences.

2.1. Ovaries

At a gestational age of 3 months, buffalo ovaries appeared as oval or spindle-shaped symmetrical thickenings just cranial to the anterior end of the differentiating Müllerian ducts and attached to the caudo-lateral borders of the kidneys. The final pelvic position of the ovaries was reached by the end of the 6th month and the right ovary was slower to descend than the left [29]. According to Dyce et al. [2], the ovaries are solid, ovoid and feature uneven surface due to the projection of follicles and corpora lutea. The cranial portion of each ovary is united to the broad ligament of the uterus by mesovary [2]. Besides the macroscopic difference [30], the ovaries of buffaloes are more elongated [15] and more firmly secured by the mesovarium compared to cattle ovaries [15,31] (Fig. 3). According to Carvalho [31], this rigid connection presented by buffaloes can hinder the movement of the ovaries during ovulation, because the mesovarium is responsible for the movements of the female gonad [3]. The lower mobility of the ovary could affect the uptake of the oocytes by the oviduct fimbriae and thus influence the recovery rate of these gametes. However, physiologically, these females should not present problems in capturing the female gametes, because both species when subjected to timed artificial insemination protocols have satisfactory conception rates [31]. The follicles on buffalo ovaries appear similar to those in cattle and the corpus luteum is considered to be more embedded in the ovarian stroma in many buffaloes [11,26] although a few may present well developed CL projecting on the ovarian surface (Fig. 3).

Figure 3. The ovarian structures (follicles, corpus luteum) on the buffalo ovary. (Photo courtesy Prof G.N. Purohit, Department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Sciences, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India). - To view this image in full size go to the IVIS website at www.ivis.org . -

Carvalho [31] found that the ovaries of buffaloes are thicker than those of cows, and the ovarian perimeter of cows is bigger than that of buffaloes. The smaller perimeter associated with greater thickness of the ovary of buffaloes relative to cattle shows that the gonad of buffaloes is rounder than that of the cow, as reported by Luktuke and Rao [9]. However, this result differs from that found by Sane et al. [15], who reported that the ovaries of buffaloes are more elongated than in cows. According to Shea et al. [32], if the ovaries are too wide, the fimbriae of the oviduct have difficulty in capturing the oocytes.

Notable variations in the dimensions of the ovaries occur during different phases of the estrous cycle [33]. According to Sisson [5], the weight of the ovaries of adult bovine female varies from 15.0 to 20.0 grams. Settergreen [22], working with Swedish Highland heifers, noted the weights of ovaries between 3.6 to 5.5 g. Carvalho et al. [20] obtained the weights of 8.9 ± 0.9 and 4.6 ± 0.4 g for the right ovary and 7.1 ± 0.7 and 5.4 ± 0.4 g for the left ovaries of cows and Murrah buffaloes, respectively, and both ovaries from cattle were heavier compared to the ovaries of buffaloes. According to some authors [9,15,27], in adult buffaloes, ovarian weight ranges from 0.5 to 10.9 g and heifers show an average weight of 3.4 to 3.6 g [11].

Carvalho [31] found smaller ovarian volume and higher fimbriae area for buffalo females compared to cattle. Thus, as the uptake and transport of oocytes depend on the interaction between the ovary and oviduct fimbriae among others [3], the larger the area of fimbriae and lower the volume of the ovary, the greater must be the capture of the oocytes by the oviduct.

1.3.2. Oviduct

The length and degree of circumvolutions of the oviducts guarding intimate anatomical relationship with the ovaries show variations between the domestic species [3]. The oviducts are tortuous structures that bilaterally extend from the ovary to the uterine horns. They have four distinct segments: the fringe like fimbriae; big orifice in the funnel-shaped abdominal opening near the ovary- the infundibulum; the more distally dilated - ampulla – and the more narrow thin wall segment that extends caudally connecting the oviduct with the uterine horn lumen- the isthmus [34]. The thickness of the musculature in the oviduct increases from the ovarian to the uterine end of the oviduct [3].

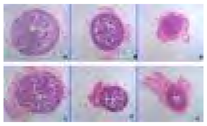

Figure 4. Histological oviducts of buffaloes and cattle. B = buffalo; C = cow. From left to right: infundibulum, ampulla and isthmus. Bar = 10 μm. - To view this image in full size go to the IVIS website at www.ivis.org . -

The characteristics of the smooth muscle of the oviduct - internal circular layer and outer longitudinal - checked in buffaloes and cows by Carvalho [31] (Fig. 4) are in agreement with literature data for Nellore cows [35] and mammals in general [3,36].
The fimbriae according to Hafez and Hafez [3] are a segment of the oviduct, while Priedkalns [34] considers them as fingerlike projections of the infundibulum. The fimbriae are shorter and less extensible in cows than in mares [5] and are free, except at one point in the upper pole of the ovary, which ensures its approach of ovarian surface [3]. The fimbriae area of buffaloes is greater than that of cows (12.51 ± 0.98 vs. 5.82 ± 0.68 cm^2, respectively) [31].

The infundibulum contains numerous secretory cells and maintains contact with the peritoneal cavity through the oviduct ostium [4]. It is thicker than the ampulla and the isthmus, and is included in the ovarian bursa [37], which in ruminants is wide and open [3]. This structure consists of thin peritoneal fold of the mesosalpinx, linked in the upper part of the ovary [3,5]. A study of multiparous buffaloes [9] found lengths of 6.6 and 7.1 cm and widths of 3.2 and 3.3 cm for left and right infundibulum, respectively. According to El-Sheikh and Abdelhadi [23], the thickness of the infundibulum in Egyptian buffalo cows ranged from 0.5 to 0.6 cm. Danell [11] found that the infundibulum diameter of Surti buffalo heifers varied from 0.2 to 0.9 cm. Changes in the infundibulum were recorded in relation to stage of reproductive cycle in buffaloes [38]. During the follicular phase the tunica mucosa of the infundibulum were highly branched occupying greater part of lumen whereas during the luteal phase cytoplasmic protrusion and lymphocytic infiltration were marked in the ampulla region [38].

For Menezo and Guerin [4], the ampullae are the real secretory portion of the oviduct. This segment corresponds to half the length of the oviduct and merges with the isthmus [3]. The isthmus has a thick wall with a more developed muscular layer [1,4] - which is tortuous, small and well defined [2]. According to Leese [36], from the infundibulum to the isthmus there is a reduction in the proportion of mucosal/muscle layer. In domestic ruminants, the junction of the isthmus to the uterine horn is not as sudden as in the mare [4], because the extremities of the oviducts are spiky, and the ostium of the oviduct is large and infundibuliform [5].

The length of oviducts of adult cattle varies from 11.0 to 33.9 cm [5,17,18,20,39] and the oviduct length of heifers ranges between 7.2 and 22.8 cm [17]. In adult buffalo, the oviduct length varies from 12.5 to 42.8 cm [7,15,20,23,27,28]. Carvalho [31] found that the oviduct lengths and uterine horn were similar between buffaloes and cattle. However, Sane et al. [15] found that the cattle reproductive system is slightly longer than that of the buffalo. As mentioned above, according to Danell [11], there is a positive correlation between body weight and measurements of the reproductive tract. The buffaloes used by Carvalho [31] were heavier than the cows, and Sane et al. [15] worked with European cows, which are heavier than the Indian buffaloes used by these authors. Thus, the difference between the results obtained by Carvalho [31] and Sane et al. [15] could be justified by the weight of the animals.

1.3.3. Uterus

In most species the uterus comprises bilateral horns connected to oviducts, a body and the cervix, which joins the vagina [34]. Sisson [5], reports that the uterus of adult cows is situated almost entirely within the abdominal cavity. The non-pregnant uterus of buffaloes is in the pelvic cavity.

The uterine horn of female buffaloes is less extensible and elastic than that of cows, because the intercornual ligament first unites the two horns from its base [28]. According to Sane et al. [15], who worked with multiparous Murrah buffaloes, the intercornual ligament of this species is more strongly attached than in cattle. This peculiarity, together with the rigid muscles of the uterus, makes the horns more tightly downward curled than in cattle (Fig. 1) [40].

The length of adult cattle uterine horns varies from 16.0 to 40.6 cm [2,5,16-18,20] and for heifers, the dimensions are 11.1 to 34.2 cm [17]. In female adult buffalo, the uterine horn length varies from 10.0 to 64.5 cm [7,15,20,23,27,28].

The uterine body of the adult female cattle measures 2.5 to 4.0 cm in length [2,5,19,20] being larger than that observed for buffaloes, ranging from 0.5 to 4.8 cm in length [7,15,20,23,27,28]. Vittoria [30] recorded the dimensions of the uterine body to vary from 0.8 to 1.5 cm in Mediterranean buffalo.

The cervix in buffaloes is comparatively shorter and narrower [7,15,20,23,27,28,40] than in cows [5,19-21]. The number of cervical rings is also lower in buffaloes than in cattle [13,18,25] varying from one to five, with an average of three rings [41]. According to Sane et al. [15], the fewer rings explain the incomplete cervix orifice dilation of the buffalo at estrus when compared to cattle. However, this was not observed by the author’s group. This also explains in part the improper cervical dilation at parturition as an infrequent cause of dystocia in buffaloes compared to cattle [42]. Age related histo-morphological changes have been recorded in the buffalo cervix [43]. The lamina epithelialis of the cervix was simple cuboidal in neonatal calves which transformed into mucinogenic simple columnar in adult buffalo [43].

The author found that the cervix diameter in Murrah buffalo heifer is small to the point of not allowing the passage of the conventional semen pipette used for adult cattle and buffaloes (unpublished personal observation), which precluded the artificial insemination of this animal category. Currently, it has become possible and feasible to use the semen pipette and sheath used for sheep and goats in Murrah buffalo heifers. Using the smaller diameter pipette in Murrah buffalo heifers, it was possible to inseminate 100% of animals passing through the entire length of the cervix [44].

The arterial pattern of uterus in buffaloes has been explained previously [45-47]. Similar to cattle the bubaline uterus is mainly supplied by uterine artery which divides into primary, secondary and tertiary branches in the broad ligament [47]. At the mesometrial border the artery is divided into several branches and in the uterine wall appears as coiled and tortuous encircling the uterine horn [47]. The pattern of vascular anastomosis observed in buffalo differed from that of the cow. The uterine branch of vaginal artery showed a prominent anastomosing branch at the level of the body of the uterus [47]. The ovarian artery gave one prominent anastomosing branch to the uterine artery and arteries on either side of the uterus were inter-connected with prominent anastomosing arches at the level of the body of the uterus [47].

1.3.4. Vagina and External Genitalia

Vaginal length in buffaloes may vary from 13.0 to 30.0 cm [7,15,20,28] whereas in cattle the measured dimensions vary from 17.5 to about 40.0 cm [5,19-21]. The vagina is smaller [20,40] and less tight [38] in buffaloes as compared to cattle [40]. According to Dyce et al. [2] the dimensions of the ruminant vagina in the passive state has relatively little importance, because the organ is capable of great expansion, both in longitudinal and in transverse directions.

The vestibule, vulva, clitoris, and vestibular glands form the external genitalia [3].

According to Hafez and Hafez [3], the junction of the vagina and vestibule is marked by the external urethral orifice and often by an overhang (the vestige of the hymen). Vittoria [30], reports that the buffalo has a characteristic morphology related to the position of the urethral opening in the vestibule. The author mentions that the structure opens at the top of a relief papillary of mucosa approximately 1 cm long, placed between two parallel folds of the mucosa. Due to this peculiarity, when the buffalo contracts the pelvis, usually in response to obstetric procedures (eg., pregnancy diagnosis), this orifice is exposed through the vulva.

The vulva in buffaloes is similar in length to that of cows, it is extremely pigmented and presents the clitoris at its ventral commissure [30]. According to Agarwal and Tomer [40] the buffalo vulva is elongated and has wide apart vulvar lips compared to cattle (Fig. 5). The lengths of the vulva of buffaloes and cattle vary in different studies (Table 1 and Table 2). The muscles of the vulva in the buffalo include constrictor vulvae and constrictor vestibuli.

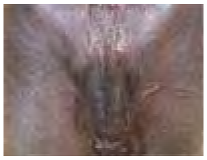

Figure 5. The external genitals of a buffalo. (Photo Courtesy Prof G.N. Purohit, Department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Sciences, Bikaner Rajasthan, India). - To view this image in full size go to the IVIS website at www.ivis.org . -

2. Anatomy of the Male Buffalo Reproductive Tract

2.1. Importance of Knowing the Anatomy of the Male Reproductive System

In order to safely perform the breeding soundness evaluation in bulls, it is crucial to know the anatomic-physiologic characteristics of the male reproductive system. Such information allows the veterinarian to understand the interrelations of the organs and to differentiate physiologic conditions from possible pathology onset [48]. Since males are responsible for 90% of a herd's genetics, choosing more fertile and precocious bulls is highly important. The basic criterion in assessing normality of the reproductive tract follows a rigorous evaluation of the reproductive organs regarding their position, shape, dimensions, symmetry, consistency, and relation with other organs. Aspects intrinsic to the animal, such as breed and age, are important during the assessment, besides environmental factors such as nutritional and sanitary management. One example of how important the anatomic knowledge is for selecting bulls is the occurrence of testicular hypoplasia, a highly heritable genetic reproductive abnormality that significantly impacts herd fertility [49]. Testicular hypoplasia can be identified by the reduction in gonad size and volume, by changes in testicle consistency, and by a functionally compromised parenchyma, which consequently reduces both the amount and quality of gamete production [50,51]. Thus, knowing the morphological aspects of the genital system enables one to identify the organic changes and their relations with subfertility and infertility, as well as adopting therapeutic measures aiming at increasing animal reproductive efficiency in case the pathology is treatable and reversible.

2.2. Factors Involved in Male Gonad Embryology

The male gonads originate embryologically from two cell types: the primordial germ cells (PGCs) and the somatic cells. PGCs will form male gametes, while somatic cells will form the somatic gonad blastema, which is responsible for PGC nutrition and for forming Sertoli and Leydig cells. In the gastrulation phase, PGCs migrate from the yolk sac wall, which is external to the embryonary structure. Attracted by chemotactic factors, these cells move through the embryo's hindgut epithelium, enter the mesenchyme of the dorsal mesentery, and reach the genital ridges by amoeboid movement. By the end of the migration phase, with the concomitant multiplication, PGCs differentiate into gonocytes and these into spermatogonia, which are able to renew and replicate and will form the male gametes [52,53].

The sex determining region of the Y chromosome (SRY gene) is responsible for beginning the sequence for the male development of an embryo. Once activated, the SRY gene stimulates the testis-determining factor (TDF) synthesis in the sexual sex cords in the still undifferentiated gonad. As a response, the pluripotent coelomic epithelial cells, which multiply and interconnect around the PGCs, differentiate into Sertoli cells [53]. Sertoli cells are responsible for PGC nutrition and for secreting the anti-Müllerian hormone (AMH) or Müllerian-inhibiting substance, which ensures the atrophy of the Müller paramesonephric ducts and the consequent development of the male reproductive system. Meanwhile, the mesonephric cells give origin to the gonadal cords, wrapped in peritubular myoblasts, that are differentiated into testicular cords and then into seminiferous tubules [52]. The testicular cords enter the medulla and merge among themselves and with the mesonephric tubules to form a network of canals in the testicular mediastinum, called *rete testis*.

The *rete testis* receives the gametes and directs them to the efferent duct, which is the communication path between the testicle and the epididymis. The deeper and more coiled portion of the testicular cords converge towards the *rete testis*. The testicular cords remain filled until puberty, when they begin to exhibit lumen and, under genetic and hormonal influence, acquire the ability to produce gametes. The efferent duct originates from the union of the *rete testis* with the mesonephric duct. The latter, under the action of testosterone, undergoes conformational changes and forms the epididymal duct. The intra-testicular septa and the tunica albuginea, responsible for gonad lobulation and support, respectively, originate from the testicular cord mesenchyma, which also plays the role of differentiating into interstitial cells, later known as Leydig cells.

Histomorphological studies on post-natal development of two accessory sex glands in the buffalo [54,55] revealed that from birth up to 6 months of the age the bulbo-urethral [54] and vesicular glands [55] were not fully developed and the two attained proper morphology and functioning near puberty.

2.3. External Organs

Due to the phylogenetic proximity between bovines and buffaloes, bubaline morpho-functional studies are usually conducted comparatively with bovines as reference. Overall, the reproductive morpho-functional characteristics in bubaline bulls are similar to those of bovines (Fig. 6), although all the component organs are smaller in buffaloes [56-58]. Externally, the male reproductive system is made up of the scrotal sac, testicles, epididymis, part of the spermatic ducts, foreskin, and penis, while inside the abdominal cavity and the pelvis are the accessory sex glands (ampullae of the deferent ducts, seminal vesicles, prostate, and bulbourethral glands), which are crucial for seminal fluid production. The anatomic-functional particularities found in buffaloes are described next.

Figure 6. Schematic diagram of male reproductive tract of buffalo. (Photo courtesy Prof G.N. Purohit, Department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Sciences, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India). - To view this image in full size go to the IVIS website at www.ivis.org . -

2.3.1. Scrotal Sac

The scrotal sac, also known as scrotum, is a sac-like structure partially divided by a septum, which forms the right and left scrotal compartments. Each compartment holds the set of organs made up of the testis, epididymis, spermatic cord, and part of the deferent ducts. The muscle-skin part of the scrotum favors the thermal exchange mechanisms allowing testicular temperature to be maintained up to 6°C below the body’s internal temperature for spermatogenesis to take place normally [59]. The scrotum has lean skin, with little subcutaneous fat, has sweat glands and little hair. Its muscle portion is called tunica dartos which, along with the cremaster muscle, promotes contraction and relaxation of the scrotal sac. This movement allows the gonads to be closer to or farther from the abdominal wall, thus favoring thermal exchange. The tunica dartos connects to the common tunica vaginalis and to other fixation structures to originate the proper ligament of the testis and the ligament of epididymis, which bind these organs to the inner portion of the scrotal sac [60]. In buffaloes, the scrotal sac is positioned closer to the inguinal region and is smaller and less pendulous than in bovines (Fig. 7) due to the slight funneling in the region corresponding to the spermatic cord [56]. In one study on Murrah buffalo bulls the scrotum was oblong in 69.77% of bulls whereas it was square in 20.93% and overlapping in 9.30% of buffalo bulls studied [61]. Problems in the insertion of the proper ligament of the testis can cause rotation at different degrees and oblique positioning of the testes, both inappropriate and followed by anomalous rotation of the scrotal sac.

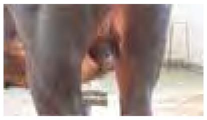

Figure 7. The scrotum and testis of a Surti buffalo bull. (Photo Courtesy Dr. Mitesh Gaur, Senior Assistant Professor, Buffalo Farm, Livestock Research station Vallabhnagar, Udaipur, Rajasthan University of Veterinary and Animal Sciences, Bikaner Rajasthan India). - To view this image in full size go to the IVIS website at www.ivis.org . -

The clinical evaluation of the scrotal sac must not be neglected given its close relation with the testicles. Overall, changes in the scrotal conformation indicate testicular or systemic changes, precursor of subfertility or infertility. The most commonly found scrotum pathologies are contusions, lacerations, dermatitis, infectious processes, hydrocele, and hematocele, which initiate inflammatory settings and a subsequent rise in temperature, causing temporary testicular degeneration [62]. Besides these, ectoparasites may infest the scrotal sac skin, mainly in regions with hotter, more humid climate.

Other changes, though of genetic origin, may impact the scrotal sac and modify its position or conformation. One such example is the bifid scrotum, characterized by the presence of a ventral-dorsal fissure occurring along the median raphe of the scrotum, of variable extension [63,64]. A study conducted with buffalo bulls raised in the field, reported that 13.1% of the animals assessed had some sort of scrotal sac pathology, with the highest prevalence being of bifid scrotum (7.8%), followed by skin disorders (4.2%) [65]. A similar study found that 25.2% of the males investigated had abnormalities related to the positioning of the scrotal sac, the most common ones being the occurrence of longitudinal scrotal torsion to the right, testicular asymmetry with volume reduction, and horizontal-ventral positioning of the scrotum, with the testis riding high in the scrotum [66].

2.3.2. Testis

Testicles are the organs hosting male gametogenesis and, therefore, are the most important ones in the reproductive context. These structures are oval shaped, oblong, latero-laterally flattened with medial and lateral faces, free and epididymal margins, and with dorsal and ventral extremities. Compared to taurine breeds, buffaloes have smaller, lighter testicles that develop more slowly [67]. Buffaloes with body weight between 400 and 600 kg have an average testicle weight of 108.7 g, while in bovines of similar bodyweight, gonads weigh approximately 160 g [68,69]. In young buffaloes, the right testicle measures on an average 8.83 x 4.64 cm (length x width) while the left testicle measures 8.77 x 4.58 cm [68,70].

Testicles are surrounded by a dense connective tissue capsule called tunica albuginea. More externally, they are surrounded by the tunica vaginalis, of peritoneal origin, which extends itself, crosses the inguinal canal, and binds to the scrotal sac. Inside the tunica vaginalis there is also the spermatic cord connected to the testicle and epididymis. Internally, the gonads are divided into two portions. The exocrine portion is made up of seminiferous tubules, whose product is the spermatozoon, a highly specialized cell capable of fertilizing the female gamete. In buffaloes, the seminiferous tubules take up 82% of the testicular space [71]. Meanwhile, the endocrine portion is represented by the intertubular space, where Leydig cells are found. These are responsible for secreting testosterone and dihydrotestosterone, which are indispensable hormones for gametogenesis and for the manifestation of secondary male sex characteristics.

The seminiferous tubules are located in lobules formed from the emission of septa from the tunica albuginea towards the center of the testicles. The tubules are extremely coiled cylindrical canals, which interconnect with the loose tissue of the mediastinum connected to the *rete testis*, to where the cell content from the spermatogenic cycle is taken. From the *rete testis*, the male gametes go to the efferent duct and from there to the epididymis. In adult animals, each seminiferous tubule is made up of the germinal epithelium, besides myoid cells (which provide contractibility to the functional unit) and Sertoli cells (which nourish and support the epithelium). The intratubular space is filled with blood vessels, lymph vessels, nerves, and several cell types, with the prevalence of Leydig cells [3,72]. Histologically, the seminiferous tubules may vary in their cell populations due to the animal’s age and consequent reproductive status [71,73,74].

In physiological conditions, 24-month-old buffalos produce on average 970 million spermatozoa per testicle every day, which corresponds to 13.26 million spermatozoa produced per gram of testicle in a single day [74]. After being produced, the spermatozoa are stored and, depending on the animal’s mating regimen, will be ejaculated or partially excreted in the urine. The total duration of spermatogenesis in buffaloes is 38.7 days, with an overall rate of spermatogenesis of 74 for the species, i.e., for each type A1 spermatogonium present in the seminiferous epithelium, 74 spermatids are formed by the end of the process.

The testicular nerves are concentrated in the tunica albuginea, mainly in the regions surrounding the medial and lateral faces of the testis. Few nerves are found in the testicular parenchyma, running along the vessels that nourish the seminiferous tubules [75]. The testicular artery, along with blood vessels, lymph vessels, and nerves, form the spermatic cords, which are vital for the organs’ functioning. In buffaloes, the testicular artery may have a varied number of ramifications, but, overall, the artery is split at the cranial and caudal branches, which also ramify, with either branch taking equal part in testicle vascularization [76].

Testicle development in buffaloes is slow and gradual until 14 months of age. Between five and 15 months of age, the gonads grow slowly and the scrotum perimeter grows approximately 0.28 cm a month. Between 15 and 25 months of age, the scrotum perimeter grows 0.91 cm a month. Between 25 and 38 months, the gonadal parenchyma stabilizes, peripheral testosterone levels increase (from 53 to 83 ng/100 mL), and spermatogenic activity takes place [77,78]. Nevertheless, spermatogenic activity may begin between 18 and 24 months of age and, when well-managed, bubaline males may have reproductive activity at 24 months of age [79], with gamete production stabilizing at 36 months [80]. It is common for bubaline bulls to stabilize their body and testicular growths at 36-48 months of age when they reach 650-750 kg, an indication of maturity [79]. It is worth pointing out that there is no full consensus in the worldwide literature regarding the beginning of puberty and sexual maturity in bubaline males, which makes it difficult to chronologically standardize the most significant endocrine and morpho-functional changes compatible with the beginning of the reproductive life. This is due to the differences inherent to the breeds and to the different nutritional and sanitary managements adopted [74,80,81].

The development of the testicular parenchyma takes place in the fetal and postnatal phases, with a marked variation in the type and amount of intra and intertubular cells depending on the animal’s age. The Sertoli cells and those of the spermatogenic lineage progressively differentiate so that, at three months of age, only gonocytes, pre-spermatogonia, and spermatogonia are present in the seminiferous tubule. Starting at six months of age, the germinal lineage is made up of gonocytes, pre-spermatogonia (whose amount gradually decreases after the first year of age and completely disappears at 36 months of age), spermatogonia, primary spermatocytes up to pachytene I, and Sertoli cells. These cell types remain in the seminiferous tubules until 12 months of age (except for gonocytes, which disappear at six months of age), varying in number so the amount of spermatocytes increases in the final phases of meiosis I while the amount of pre-spermatogonia decreases. Mature Sertoli cells appear at nine months of age and increase in number in the following months, reaching their peak starting at 21 months of age. Simultaneously, the primitive cell types decrease and completely disappear at 30 months of age [82]. Starting at 24 months, round and oblong spermatids can be found, as well as spermatozoa and cellular associations that make up all phases of the spermatogenic cycle, signaling the beginning of puberty [82]. The interstitial space also undergoes histological changes after birth. Up until three months of age, the cell types found are mesenchymal cells, fetal-type Leydig cells, fibroblasts, myoid cells, pericytes, and endothelial cells. Mature Leydig cells increase in number between 30 and 72 months of age [73], indicating an increase in testosterone production.

Due to the cell multiplication process in the testis in the first year of life, the seminiferous tubules (still called testicular cords at this phase) reach a diameter between 104.4 ± 3.5 and 131.76 ± 13.1 μm. At this time, the lumen diameter still cannot be determined because, although there is histological uniformity, there is no complete formation of germinal epithelium, which makes it possible to find cords at early spermatogenic activity and others undergoing vacuolation [74,80,81].

At 24 months of age, the seminiferous tubules are completely formed and it is possible to obtain histometric data compatible with their functional status. Testicles in 2-year-old bulls have seminiferous tubules with diameter of 156.96 ± 4.51 µm, epithelium height of 34.72 ± 2.77 µm, 79.35% of tubular volume, tubular length per testicle of 3,003.93 ± 675.5 m and 42.94 ± 12.5 m per gram of testicle. In buffaloes between four and five years old, the mean diameter of the seminiferous tubules is 245 µm while the lumen may reach 60 µm, values below those found in bovines [71].

From the ultrastructural standpoint, the spermatogenic linage cells are similar to those found in bovines. However, there are differences in the organelle dimensions, mainly during the prophase I phases (pre-leptotene, leptotene, zygotene, pachytene, diplotene, diakinesis) of meiosis I, such as oscillation of the mitochondrial volume, eight-fold increase of the Golgi apparatus, and differentiation and volume increase of the endoplasmic reticulum. The spermatogenic cycle in buffaloes takes place in six phases and there is evidence that 63% of all germinal cells disappear during spermatogenesis and meiosis, likely due to the spermatogonial degeneration and loss of cells to the interior of the lumen [71].

From the macroanatomy standpoint, some measurements of the testicles are important for their relation with testicle growth and their ability to predict gamete production. During the testicular biometry evaluations, measuring the scrotal circumference stands out because bulls with greater scrotal circumference have more reproductively precocious daughters. There is also a positive correlation between testicular and body development in bulls, upon which the beginning of puberty and sexual maturity depend. For example, over time, the animals grow, gain weight, and have larger thoracic circumference and depth [83]. The scrotal circumference follows this growth (Fig. 8) and has been associated with some measurements such as weight ($y=12.299+0.029x$; $R^2=0.92$; y=scrotal circumference in cm and x=live weight in kg) and age ($y=16.943+0.279x$; $R^2=0.69$; y=scrotal circumference in cm and x=age in months) [83]. The scrotal circumference is also associated with important semen quality characteristics such as sperm concentration and motility, gross motility, and percentage of normal cells in the ejaculate, besides serum testosterone levels [84,85].

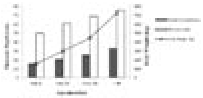

Figure 8. Evolution of the scrotal circumference development in buffalo bulls as a function of age, body weight, and thoracic depth (adapted from Gonçalves et al. [83]). - To view this image in full size go to the IVIS website at www.ivis.org . -

Compared to taurine bovines, testicular development is slower in buffalo bulls since the former can have scrotal circumference of 30 to 33 cm between 12 and 24 months of age. This value is only found in buffaloes over 30 months old that are kept under appropriate nutritional and sanitary conditions [55,64,77-79,85-87]. Data on buffalo's scrotal circumference, from birth to sexual maturity, showing their gonad evolution, are shown in Table 3.

2.3.3. Epididymis

The epididymis is the organ responsible for nutrition, maturation, selection, transport, and storage of spermatozoa. Along the epididymal transit, the male gamete acquires motility ability and undergoes morphological, molecular, and metabolic changes needed for it to have fertilizing potential. The epididymis in adult bulls is 13 cm long and weighs on average 18 g [70].

The epididymis is a tubular-shaped organ located medially to the testicle and is formed by three regions, namely the caput, corpus, and cauda. In a recent study the dimensions of the corpus epididymis in buffaloes during the mating and non-mating seasons were 393.99 ± 8.97 and 343.64 ± 10.42 µm whereas those of the cauda epididymis were 675.81 ± 15.52 and 687.23 ± 18.01 µm during the mating and non-mating seasons respectively [90]. These regions connect to the dorsal, ventral, and caudal portions of the testis by means of a fibrous fixating tissue [60]. Externally, the epididymis is surrounded by a tunica of connective tissue under which is a muscle layer. The epididymal duct has a very visible basement membrane with a pseudostratified columnar epithelium and sterocilia on the apical surface.

The epithelium of the epididymal duct is made up of principal cells, basal cells, apical cells, and intraepithelial leukocytes. The principal cells are responsible for secreting and absorbing activities. The basal cells are the second most numerous cell groups in the epididymis, whose role is likely to serve as reserve cells for the renewal of the epididymal epithelium. The apical cells also make up the epididymal epithelium, although their role is not yet clear. In buffaloes, this cell group spreads along the epididymal duct, more markedly in the caput epididymis, and seems to act on the hydroelectrolytic balance and in the acidification of the epididymal fluid. The intraepithelial leukocytes are migratory cells common in the epididymal epithelium and include both lymphocytes and macrophages involved in the induction of immune tolerance of the epididymis [91]. In the peritubular space, small-bore blood vessels are associated to mastocytes, which secrete angiogenic and chemotactic substances that control vascular permeability [92] and induce the migration of other cells from the immune system into the organ’s interstitium and, subsequently, into the epididymal epithelium [91].

Arterial blood is supplied to the epididymis by successive ramifications of the testicular artery, which originate vessels of different bores and spread differently across the three epididymal regions. The proximal branch of the testicular artery extends through the tunica albuginea and irrigates the caput and corpus epididymis. Meanwhile, the distal branch feeds the cauda of the organ. Inside the epididymis, these branches split into interlobular arteries that irrigate the medial and lateral sides of the organ and then originate the arterioles making up the network of venous capillaries that flow into the caput epididymis and penetrate the fibromuscular layer of the efferent ducts. The network of venous capillaries flows into the veins of the caput, corpus, and cauda, which meet the veins of the tunica and confine the pampiniform plexus. The endothelium of the venous capillaries has fenestrations of different shapes and sizes that enable them to connect to the lymph vessels so as to ensure secretion, absorption, and circulation of vital biomolecules for spermatic maturation [93].

The propulsion of the immotile spermatozoa from the caput, corpus, and cauda of epididymis occurs by means of spontaneous phasic contractions of the surrounding smooth muscle layers (tunica muscularis) [94]. The transport of the sperm through the buffalo epididymis is characterized by pendular movements providing stirring of intraluminal content [95]. Buffaloes produce an abundance of constitutive NOS (endogenous endothelial nitric oxide synthase - NOS I and NOS III) in the epithelium of the caput epididymis during the winter period only. The capacitation and motility of the spermatozoa can probably be modified by the presence of nitric oxide in the caput epididymis during the winter season [94], when buffaloes are more likely to reproduce.

Spermatozoa transit along the buffalo epididymis takes 9.3 days, being 3.2 days the time needed for them to pass along the caput and the corpus and the remaining 6.1 days for them to pass along the cauda epididymis [96]. This time may be shortened by more frequent mating or semen collection through artificial means.

2.3.4. Spermatic Cord

The spermatic cord, also called spermatic funiculus, is formed by the set of tubular structures grouped in a cord-like shape that come from the abdomen and reach the testicles. The spermatic cord is formed by the deferent ducts, arteries, pampiniform plexus, lymph vessels, nerves, and cremaster muscle. All these elements are surrounded by the tunica vaginalis, cross the inguinal canal, and reach the testicles to perform circulatory and thermoregulatory roles, conduct nerve impulses, and transport gametes.

The deferent ducts (or deferent canal) comprise a tubular structure that conducts the semen stored in the epididymis to the urethra. The pampiniform plexus is formed by a network of venous vessels that surround the testicular artery, which derives from the abdominal aorta artery. Such anatomic disposition enables a larger contact surface between the vessels and increases countercurrent heat exchange due to the temperature difference between the bodily blood, brought in by the artery, and the blood from the testicles, carried by the plexus. This way, the blood reaching the testicular parenchyma is 4 to 5°C below the body temperature, an important factor to maintain regular spermatogenesis. Environmental heat variations activate the lumbar sympathetic nerve block, which commands the contraction or relaxation of the cremaster muscle, responsible for keeping the testicles closer to or farther from the abdominal wall, thus allowing for the regulation of gonad temperature [60,97]. The spermatic cord can develop pathologies such as varicocele, which may result in subfertility due to a failure in testicular thermoregulation.

2.3.5. Inguinal Canal

The inguinal canal is a muscular aponeurotic structure that forms an oblique passage from the caudal portion of the abdominal wall that begins at the deep inguinal ring and ends at the superficial inguinal ring. The inguinal canal allows the testis, epididymis, and spermatic cords to pass from the abdominal cavity into the scrotal sac. The deep inguinal ring is formed from the free caudal edge of the internal oblique muscle and by the inguinal ligament and interconnects the inguinal canal to the abdominal wall. The superficial inguinal ring is formed from the slit in the aponeurosis of the external oblique muscle located near the pubis and interconnects the inguinal canal to the subcutaneous groin tissue.

The inguinal canal is structurally fragile and, therefore, the organism resorts to passive and active mechanisms to strengthen that structure and prevent abdominal gut herniation. The passive mechanisms involve the placement of perpendicular fibers at the apex of the superficial inguinal ring in order to prevent ripping and to increase the ring’s diameter. Moreover, the fact that the inguinal rings are not juxtaposed and the presence of the tendon provides greater stiffness to the canal walls. The active mechanism works through the contraction of the internal and transverse oblique muscles that occlude the canal opening, which remains virtually shut [98].

Anatomic changes to the inguinal canal may cause the animal to have pathological conditions, such as ectopic testis, that occurs when one of the testicles deviates during its descent from the *gubernaculum testis*, usually due to changes in the structure of the inguinal canal. There may also be uni or bilateral cryptorchidism, an abnormal condition in which one or both testicles, respectively, cannot be found in the scrotal sac. In this case, the testicle is held in the abdominal cavity or in the inguinal canal. In spite of the etiology of cryptorchidism being primarily attributed to genetic expression anomalies and to the insufficient production of hormones involved in the mechanism of testicular migration from the abdominal cavity into the scrotal sac, the narrowing or fibrosis of the inguinal ring may contribute to the manifestation of this pathology [99,100]. In addition, the weakening of the inguinal rings, allied to the increase in intra-abdominal pressure, may favor gut herniation through the internal inguinal canal and lead to an inguinal hernia setting with asymmetric and painless scrotal swelling [101].

2.3.6. Penis

The penis is the copulatory organ and is of fibroelastic nature in buffaloes, a species in which ejaculation is intravaginal. In adult buffalo bulls, the average penis length is 80.15 cm, measured from the beginning of the sigmoid flexure to the free end, while the average thickness is 1.95 cm [64]. The penis has a cylindrical shape and is anatomically divided into three regions: crura (or root), body, and glans. The crura and the base of the penile body make up the intrapelvic penis, while the body and the free end, which contains the glans, make up the extrapelvic penis [60].

The penis is formed by muscle tissue besides the cavernous and spongy tissues, which are erectile and surrounded by the tunica albuginea, made up of fibroelastic connective tissue and muscle cells [102]. The internal elongations from the tunica determine the cavitary architecture present in the organ’s body. In buffaloes, the fibroelastic origin provides the penis a firm consistency and limits the increase in volume of the organ during erection. Great amount of elastic fibres (65.41% for corpus spongiosum and 51.12% for corpus cavernosum) suggest that these fibres have an important role in penile erection in the buffalo [102]. The penis root originates from two foundations, called *crura penis*, that come from the lateral ends of the ischial arch meeting below. It is lined with ischiocavernosus and bulbo-spongiosus muscles [103].

The body of the penis is formed from the confluence of the ischial arch pilars. It has four surfaces: The dorsal surface, which harbors large arteries, veins, and nerves; the ventral surface, which contains the urethra; and two innervation-rich side surfaces. It is made up of an even number of parallel corpora cavernosa on the right- and left-hand sides of the organ. The urethral groove, surrounded by the corpus spongiosum, is found in the ventral portion of the penis body. These tissues are formed by fibroelastic lacunae that are filled and emptied during erection, by muscle cells, and by numerous blood vessels that, when there is a sexual stimulus, guarantee the increase in blood flow towards the interior of the corpus cavernosum and leads to a certain ingurgitation and expansion of the organ. At the moment of relaxation after copulation, the spaces of the corpora cavernosa and corpora spongiosa are emptied [104]. The corpus spongiosum, originating at the base of the penis and finishing at the organ’s glans, is also filled with blood and completes the erectile process.

The ischiocavernosus muscles originate at the medial portion of the ischial tuberosity and enter the base of the penis. Their contraction promotes pumping and retention of blood inside the corpus cavernosum, ensuring the erection. The bulbospongiosus muscle surrounds part of the corpus spongiosum of the urethra and extends until the bulbourethral glands. Its contraction fills the corpus spongiosum during erection. The penis smooth retractor muscle originates at the caudal vertebrae and enters the ventral and sinuous portion of the penis, called sigmoid flexure. This flexure is S shaped and, through contraction and relaxation movements of the penis retractor muscle, leads to retraction or erection, respectively. This mechanism is quite relevant for penile erection and exposition to take place in ruminants given the limited ingurgitation ability of the corpus cavernosum in these species [3,105]. The muscles of the penis in the buffalo bull include ischiocavernous and retractor penis muscle [106].

The glans has a tapered shape, is dorsoventrally flattened, and has a slight curvature and a groove, whose end has an orifice called urethral ostium [105,107]. At the moment of the copula, the glans undergoes a conformation change so that, after its removal from the vagina, it assumes a spiral shape [3].

Blood is supplied to the penis by ramifications of three main arteries: the internal pudendal, external pudendal, and obturator. The internal pudendal artery splits into bulb, deep, and dorsal arteries of the penis, the latter responsible for irrigating all the dorsal extension of the body of the penis, while its numerous subdivisions irrigate the foreskin. The external pudendal artery originates the cranial artery, which irrigates the tunica albuginea. Satellite veins of the large arterial vessels drain the venous flow from the erectile corpora and the penis tissue. The corpus cavernosum and corpus spongiosum are drained through different paths. The venous blood from the corpora cavernosa returns to the systemic circulation by means of the deep veins, while the venous flow from the corpus spongiosum and the bulb of the penis is drained by the system of dorsal veins and their lateral branches [108,109].

The penis innervation is composed mainly of the pudendal nerve, which splits into the dorsal nerve of the penis and superficial and deep perineal nerves. The dorsal nerve is responsible for the nervous stimulation of the whole copulatory organ and is located along the lateral dorsal surface of the penis so as to guarantee the supply to the intra and extrapelvic portions. To that end, the dorsal nerve branches near the tunica albuginea carry nervous impulses to the body and to the glans, the latter irrigated by numerous terminal branches. The superficial perineal nerve is responsible for supplying the foreskin and the scrotum, while the ramifications of the deep perineal nerve provide the stimuli to the ischiocavernosum and bulbospongiosum muscles [103].

The erection process takes place in face of visual, olfactive, and tactile stimuli that activate the parasympathetic nervous system. This systems triggers circulatory responses such as the dilation of the dorsal and deep arteries of the penis, increasing their blood supply. The arterial blood pumped is retained in the corpora cavernosa by the contractile action of the ischiocavernosum muscle, thus causing an increase in internal pressure and the consequent penile elongation and dilation that characterizes the erection. When the ischiocavernosus muscles contract, they obstruct the veins that drain the corpora cavernosa, maintaining the pressure established inside the penis. Meanwhile, the penis retractor muscle relaxes and loosens the sigmoid flexure, releasing the copulatory organ outside the foreskin [108].

The penile apical ligament is a widening of the tunica albuginea, beginning at the distal curvature of the sigmoid flexure and made up of collagen fibers. The apical ligament lines the exterior of the dorsal portion of the penis along its length and is formed by collagen fibers that have greater elasticity on the left-hand face and little elasticity on the opposite and medial faces. Its thickness varies depending on the region of the penis, being narrower at the base of the penis and at the distal portion and wider at the points where it surrounds the tunica albuginea [110]. Physiologically, this ligament aids in the erection process by maintaining the distal portion of the penis raised, by preventing the penis from deviating at intercourse, by adapting the glans to the spiral shape, and by repositioning the external urethral ostium for the efficient semen deposition in the vaginal canal [111-113].

Even though the occurrence of penile pathologies in buffaloes is low [64,65], some changes may compromise the regular penile anatomy and, thus, the reproductive performance. These changes include lacerations, bruises, mechanical traumas, deviations (spiral, ventral, or S-shaped deviations), the presence of fibropapilomas, urethral fistulas, and loss of glans sensitivity [60,114,115].

2.3.7. Foreskin

The foreskin, also called preputial sheath, consists of the outer penile cover of cutaneous and muscular origin with a shape corresponding to the penis and located in the ventral region, between the scrotal sac and the navel. It is responsible for the mechanical protection of the male sexual organ. Buffalo bulls have shorter preputial sheath with less fur and closer to the abdominal wall (Fig. 9) when compared to bovines [48]. Moreover, a difference can be noticed among breeds and individuals regarding the position of the sheath in relation to the abdomen [3]. The prepuce was pendulous (30.43%), medium (52.18%) and tight (17.39%) in 60 Murrah buffalo bulls at sperm stations in India [116].

Figure 9. The prepuce of a Surti buffalo bull. (Photo Courtesy Dr. Mitesh Gaur, Senior Assistant Professor, Buffalo Farm, Livestock Research station Vallabhnagar, Udaipur, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India). - To view this image in full size go to the IVIS website at www.ivis.org . -

In ruminants, the release of the penis takes place, physiologically speaking, only at the copula. The foreskin assumes an oblong, thin shape that ends with a keratinized and vascularized round orifice called preputial ostium, through which the penis exposition takes place. It has distinct inner and outer linings, whose transition point is the preputial ostium. Its layered constitution provides enough mobility to cover and uncover the penis, a characteristic that must be deemed important in andrological evaluations [104].

The preputial sheath is divided into the penile region – where a connective tissue between the foreskin and the penis is found, developed as early as in the embryonary phase – and prepenile region, comprising the free part of the copulatory organ [117]. Externally, the foreskin is formed by the thin and mobile skin and by hair that serve as a mechanical barrier. The stratified pavement epithelium prevails in it and there are sebaceous glands that produce smegma [118]. The inner layer, formed from the invagination of the outer portion, is lined with mucosa and dense connective tissue arranged in longitudinal folds filled with secreting glands, blood vessels, and lymphatic tissue [119]. When these two layers are separated, the vascular complex is found from the numerous ramifications of large arteries and veins [120].

The contractile movements of the foreskin are performed by the cranial preputial (or proactor) muscles and caudal (or retractor) muscles inserted into the cutaneous, intermediary, and deep layers of the organ. The pair or preputial cranial muscles are formed from the cutaneous trunk muscle and are dorsally inserted into the preputial ostium. When they contract, they promote the forward traction of the foreskin and the closing of the ostium, preventing penile protrusion.

Moreover, they redirect the penis and the foreskin after the mount. The caudal preputial muscles irradiate from the inguinal region and fixate to the caudal portion of the ostium, below the fibers of the cranial muscles. Their connection with the inner layers is considered superficial, so that when they contract, there is traction of the outer wall of the penile region without influencing the remaining of the organ and the penis [119].

The pathologies that can affect the foreskin are quite important since they prevent the breeding animal from mating either temporarily or permanently depending on their nature and severity. Chronic preputial prolapse is characterized by the continuous exposition of the inner lamina of the foreskin, making it vulnerable to trauma that may evolve into secondary infections among other aggravating conditions. It may be congenital or acquired and related to the contractile deficiency or lack of retractor muscles of the preputial sheath [60,121]. Another predisposition factor is a long foreskin with an ostium of greater diameter.

Also an important disorder is phimosis, deriving from the narrowing of the preputial ostium and caused by genetic factors or inflammatory processes due to wounds, ulcerations, necrosis, and fibrosis that culminate with total stenosis of the preputial ostium. Balanoposthitis or phalloposthitis is the inflammatory process of the foreskin and the glans characterized by lacerations and acute pain to the animal [60,117], which interferes with fertility and may cause potential complications such as the development of paraphimosis or phimosis.

The persistence of the preputial frenulum is considered a cause for impotentia coeundi, i.e., it is a factor that hinders the mating ability. The preputial frenulum consists of connective tissue connecting the ventral part of the penis and the foreskin that, generally, begins to disappear at four months of age. Its permanence causes a limitation in penile extension and leads to penile deviation at the mount and consequent inability of intercourse. The permanent frenulum is connected to a blood vessel that stimulates the thickening of the connecting structure, thus making its rupture difficult [115,122].

2.4. Internal Organs

The internal male reproductive system is made up of accessory sexual glands that comprise the seminal vesicles, the ampullae of the deferent ducts, the prostate, and the bulbourethral glands. In buffaloes, the accessory glands are smaller than in bovines of similar age, however, physiologically their roles are identical [68].

The secretions of the accessory sexual glands, allied to those from the epididymis and the testis, make up the seminal plasma. They contain several substances such as fructose, citric acid, potassium, zinc, acid phosphatase, free amino acids, and prostaglandins, which are added to the semen at the moment of ejaculation [123]. Besides neutralizing vaginal pH, the primary roles of the seminal plasma are assuring organic and inorganic molecular support to the spermatozoa for energy production, maintaining the medium osmolarity, protecting against reactive oxygen species (ROS), modulating sperm motility, and acquiring the ability of performing spermatic capacitation and the acrosome reaction [124-126]. The seminal plasma also contains proteins related to the high semen freezability such as the acid bovine seminal fluid (aSFP), clusterin, albumin, and osteopontin (OPN). On electrophoresis two additional protein bands can be seen (15-16 kDa, isoelectric point of 4.7 to 5.2) that may correspond to proteins BSP1 or BSP3 [127,128].

2.4.1. Seminal Vesicle

The seminal vesicles, also called vesicular glands, are multilobulated organs of firm consistency located dorsal to the urinary bladder, lateral to the ureter and the ampullae of the deferent ducts, and cranial to the prostate. These structures are present in even numbers, are palpable, and their size vary with animal age [60], even though they represent the largest accessory sexual gland in buffaloes.

In adult bubaline bulls, the vesicles are half the size of the organ in bovines, i.e., 8 to 10 cm long and 2 to 3 cm in diameter, with less evident lobulations [68,70]. They are covered in a capsule of fibrous connective tissue that contains smooth muscle cells and fibroblasts, which extends trabeculae that split the gland into lobes and lobules. In buffaloes, these glands are tubulo-alveolar, with a columnar-type secreting epithelium with a few scattered rounded basal cells [129]. The epithelium of the seminal vesicles is responsive to the action of sexual hormones and, thus, is more developed with greater secreting activity in sexually mature animals [130].

In buffaloes, the seminal vesicles are responsible for the production of 60% of the semen volume and are the main sources of fructose that makes up the seminal plasma. Besides secreting fructose and citric acid (elements crucial to the spermatic metabolism), the seminal vesicles also secrete sodium, potassium, prostaglandin, lipids, and important proteins for high sperm motility and semen quality. Among the latter ones, one can mention the acid protein of the bovine seminal fluid [131], osteopontin [132], and BSP1, BSP3, and BSP5, these three representing more than 60% of the total proteins of the seminal plasma [128]. The antioxidant secretions of the seminal vesicles can protect spermatozoa against the action of ROS and, therefore, play an important role in male fertility [133].

Seminal vesicles can be affected by an inflammatory process, either congenital or acquired, called vesiculitis, whose onset is caused by bacteria, viruses, fungi, or protozoa. Vesiculitis is the most common disorder in seminal vesicles and can affect up to 3.2% of breeding buffaloes [57]. This condition [57] has considerable clinical and economic relevance since it often presents subclinically and is transmissible, being detected only during the clinical andrological evaluation. For this reason, the early retirement of animals stricken with the chronic form of the disease is adopted as a solution [60]. Other congenital disorders of the seminal vesicles, such as unilateral segmental aplasia and bilateral infantilism, can be found in buffaloes [134].

2.4.2. Ampullae of the Deferent Ducts

The deferent ducts are canals that are tubular, long, and thin, of firm consistency and have thick muscle walls. Their role is to bring the spermatozoa from the epididymis to the urethra. The pelvic terminal portion of each deferent duct has a dilation called ampulla of the deferent ducts. The deferent ducts, along with the ducts of the seminal vesicles, pass under the body of the prostate and reach a protuberant structure called seminal colliculus (*colliculus seminalis*) [135], which is a component of the pelvic urethra.

In buffaloes, both the deferent ducts and the accessory sexual gland have noradrenergic innervations. Their activation causes the smooth muscles to contract and, thus, it is supposed to permit the progression of spermatozoa through the deferent ducts, besides the secretion of the contents of the vesicular glands and the prostate. Since this innervation is highly dependent of the action of estrogen, its integrity during the mating season is essential for maintaining the reproductive role of buffalo bull [136].

In buffaloes, the ampullae of the deferent ducts synthesize fructose and citric acid, besides the acid protein of the seminal fluid and osteopontin [127]. Their size varies according to the bull’s age, but in adult bulls the ampullae are on average 9.2 to 9.4 cm long and 0.64 to 0.66 cm in diameter [68]. Among the abnormalities that affect the deferent ducts, segmental aplasia is observed; its main consequence is the formation of spermatic granulomas in case there is a rupture of the epididymal duct and leakage of the spermatic content into the interstitial region [137,138].

2.4.3. Prostate

The prostate is a single gland located on the floor of the pelvic cavity between the seminal vesicles. The prostate is divided into two parts: the body and the disseminate part. In adult buffaloes, the prostate body can be 1.57 and 1.48 cm in length and width, respectively [70]. The disseminate part can be 6.7 cm long, 2.9 to 3.9 cm wide, and 2.4 cm thick [139]. When compared to bovine bulls (*Bos taurus*), the prostate is considered well developed in buffaloes, which leads to the assumption that it plays a more important role in the latter [140].

In buffaloes, the body of the prostate is small and is projected onto the dorsal surface of the urethra between the vesicular glands. The disseminate part of the prostate represents the largest portion of the organ and is concealed in the wall of the urethra and covered ventrally and laterally by the urethral muscle [135]. The disseminate part is well developed and forms a dense layer of glandular tissue around the prostatic urethra, surrounding it.

The prostate is covered in a thin fibroelastic capsule with trabeculae that segment the prostatic tissue into incomplete lobules [129]. In buffaloes, the prostate has a tubulo-alveolar architecture and the secreting epithelium of the tubules and alveoli are of simple columnar or cuboidal epithelium types [129].

The prostate secretes a whitish, milky, alkaline liquid rich in fructose, citric acid, cholesterol, proteins, and free amino acids. The prostatic liquid is secreted by the prostate and reaches the urethra via the prostatic ducts, or prostatic ductules, which open onto the floor of the prostatic portion of the urethra.

In buffaloes, the occurrence of neurotransmitter- and neuropeptide-synthesizing enzymes in the prostate tissues, as well as in the ampullae of the deferent ducts, vesicular glands, and vas deferens is much more intense during the mating season than during the non-mating season [136].

2.4.4. Bulbourethral Glands

The bulbourethral glands, also called Cowper’s glands, are small structures of firm consistency and rounded shape. They are located in a caudo-dorsal position in relation to the pelvic urethra so that their ducts converge into the urethral canal. The glands have a thick connective tissue capsule that extends fibroelastic trabeculae and also have smooth muscle cells. The trabeculae segment the organ into lobules and the intralobular stroma is supported by a reticulum rich in thin collagen fibers. The structure of the bulbourethral glands is tubulo-alveolar and their secreting terminations are lined with columnar and cuboidal epithelium, a variation that can be attributed to the differences in the cells’ secreting activity [129].

In buffaloes, the bulbourethral glands secrete mucosa prior to the copula so as to cleanse and lubricate the urethral canal, which will receive the semen [3]. This secretion removes any urine residue that may be harmful to the spermatozoa in the ejaculate. Along with the secretions of the prostate, seminal vesicles, and ampullae of the vas deferens, they are regular component fluids of the ejaculate.

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Anatomy of the Reproductive Tract of the Female and Male Buffaloes

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Table 1. Biometry of the Cow's Genital System According to Different Authors

Organ	Measure	[16] Adult Cows Nellore	[17] Adult Cows Nellore	[18] Adult Cows Undef. Breed	[19] Adult Cows Undef. Breed	[5] Adult Cows Undef. Breed	[20] Adult Cows Nellore	[21] Beef Heifers	[16] Heifers Nellore	[17] Heifers Nellore	[22] Heifers Swedish Highland
Left Ovary	Length (cm)	-	-	3.4±0.5	-	-	2.9±0.1	3.8	-	-	2.57
	Width (cm)	-	-	2.2±0.5	-	-	2.0±0.1	1.7	-	-	1.91
	Thickn. (cm)	-	-	1.6±0.4	-	-	1.2±0.1	2.6	-	-	1.61
	Weight (g)	-	-	-	-	-	7.1±0.7	9.4	-	-	4.4 (3.7-5.5)
Right Ovary	Length (cm)	-	-	3.6±0.6	-	(3.5-4.0)	3.3±0.1	3.7	-	-	2.51
	Width (cm)	-	-	2.4±0.5	(0.7-5.0)	2.5	2.2±0.1	1.7	-	-	1.84

Organ	**Measure**	**[16] Adult Cows Nellore**	**[17] Adult Cows Nellore**	**[18] Adult Cows Undef. Breed**	**[19] Adult Cows Undef. Breed**	**[5] Adult Cows Undef. Breed**	**[20] Adult Cows Nellore**	**[21] Beef Heifers**	**[16] Heifers Nellore**	**[17] Heifers Nellore**	**[22] Heifers Swedish Highland**
Right Ovary	Thickn. (cm)	-	-	1.7±0.4	-	1.5	1.4±0.1	2.3	-	-	1.53
	Weight (g)	-	-	-	-	(15.0-20.0)	8.9±0.9	8.6	-	-	4.0 (3.6-4.7)
Left Oviduct	Length (cm)	17.7 (13.4-23.0)	18.1±3.5	20.7±2.3	-	-	18.8±0.7	-	15.2 (11.2-20.4)	17.9±3.8	-
	Width (cm)	-	-	0.3±0.0	-	-	-	-	-	-	-
Right Oviduct	Length (cm)	17.6 (13.0-23.5)	23.8±4.4	20.7±2.4	-	-	18.8±0.5	-	15.4 (10.5-20.7)	24.0±4.8	-
	Width (cm)	-	-	0.3±0.0	-	-	-	-	-	-	-
Left Horn	Length (cm)	26.0 (17.7-33.7)	19.0±5.0	39.0±6.8	-	-	28.8±1.0	-	14.8 (11.1-18.5)	19.2±5.0	-
	Width (cm)	-	-	2.3±0.4	-	-	5.6±0.2	-	-	-	-
Right Horn	Length (cm)	26.0 (19.0-33.5)	33.7 6.7	39.6 7.4	-	-	29.5±1.3	-	14.6 (11.2-18.5)	32.2±6.9	-
	Width (cm)	-	-	2.4±0.6	-	-	5.7±0.3	-	-	-	-
Uterine Body	Length (cm)	-	2.3 (1.0-4.0)	-	(2.5-3.7)	(3.0-4.0)	3.0±0.2	2.3	-	1.6 (0.5-3.4)	-
Cervix	Length (cm)	-		8.0±1.4	(5.0-10.0)	10.0	8.0±0.3	11.5	-		-

Organ	**Measure**	**[16] Adult Cows Nellore**	**[17] Adult Cows Nellore**	**[18] Adult Cows Undef. Breed**	**[19] Adult Cows Undef. Breed**	**[5] Adult Cows Undef. Breed**	**[20] Adult Cows Nellore**	**[21] Beef Heifers**	**[16] Heifers Nellore**	**[17] Heifers Nellore**	**[22] Heifers Swedish Highland**
Cervix	No of Rings	-		3.2±0.7	-	-	3.8±0.2	-	-		-
Vagina	Length (cm)	-		-	(17.5-25.0)	(25.0-30.0)	35.7±1.5	25.0	-		-
Vulva	Length (cm)	-		9.6±1.2	(10.0-12.5)	10.0	-	10.7	-		-

Table 2. Biometry of Buffalo Genital System According to Different Authors

Organ	**Measure**	**[23] Adult Buff. Egyptian**	**[24] Adult Buff. Egyptian**	**[9] Adult Buff. Undef. Breed**	**[25] Buff. Non Descript**	**[15] Adult Buff. Murrah**	**[26] Adult Buff. Murrah**	**[27] Adult Buff. Jaffri**	**[28] Adult Buff. Undef. Breed**	**[20] Adult Buff. Murrah**	**[11] Heifers Surti**
Left Ovary	Length (cm)	-	2.2 (2.0-2.4)	2.6 (1.6-3.8)	2.2±0.0	3.0±0.0 (1.2-5.5)	2.3±0.1 (1.9-3.2)	3.1±0.0 (1.5-5.0)	2.5±0.7 (1.1-4.5)	2.9±0.1	2.3±0.0
	Width (cm)	-	1.5 (1.4-1.7)	1.3 (1.0-1.6)	1.6-0.3	1.4±0.0 (0.4-3.5)	2.0±0.0 (1.6-2.5)	1.4±0.0 (0.6-2.6)	1.4±0.5 (0.0.-2.8)	2.1±0.1	1.7±0.0
	Thickn. (cm)	-	1.4 (1.3-1.5)	1.5 (1.0-1.9)	1.5±0.0	1.1±0.0 (0.5-2.2)	1.5±0.1 (1.2-2.3	1.1±0.0 (0.5-2.0)	1.5±0.5 (0.6-3.0)	2.1±0.1	1.4±0.0
	Weight (g)	-	-	3.9 (2.0-7.7)	3.0±0.1	3.7±0.1 (0.5-10.9)	-	3.9±1.6 (0.2-8.6)	4.7±2.9 (1.6-13.0)	5.4±0.4	3.4±1.3
Right Ovary	Length (cm)	-	2.4 (2.2-2.6)	2.5 (1.7-3.6)	2.2±0.0	2.9±0.0 (1.1-5.0)	2.2±0.1 (1.3-2.7)	3.1±0.0 (1.5-5.1)	2.5±0.7 (1.5-4.0)	2.8±0.1	2.3±0.0

Organ	Measure	[23] Adult Buff. Egyptian	[24] Adult Buff. Egyptian	[9] Adult Buff. Undef. Breed	[25] Buff. Non Descript	[15] Adult Buff. Murrah	[26] Adult Buff. Murrah	[27] Adult Buff. Jaffri	[28] Adult Buff. Undef. Breed	[20] Adult Buff. Murrah	[11] Heifers Surti
Right Ovary	Width (cm)	-	1.6 (1.5-1.8)	1.3 (0.9-1.8)	1.6 0.1	1.4±0.0 (0.5-3.0)	1.7±0.1 (1.3-2.3)	1.5±0.0 (0.7-2.5)	1.4±4.5 (0.8-3.0)	2.0±0.1	1.8±0.0
	Thickn. (cm)	-	1.2 (1.1-1.4)	1.6 (1.1-2.0)	1.7±0.0	1.2±0.0 (0.5-2.5)	1.4±0.0 (1.3-2.3)	1.2±0.1 (2.0-0.6)	1.6±0.5 (0.7-3.4)	2.2±0.2	1.4±0.0
	Weight (g)	-	-	4.0 (2.3-6.6)	1.7±0.0	3.8±0.1 (0.6-11.1)	-	4.0±1.5 (0.6-10.2)	4.8±2.9 (1.3-12.5)	4.6±0.4	3.6±1.5
Left Oviduct	Length (cm)	26.3±0.3 (16-34)	-	21.8 (16.1-31.9)	16.6	22.4±0.1 (12.5-42.8)	23.3±0.6 (18-30)	24.5±0.3 (13-36.2)	19.5±5.1 (11-29)	20.8±0.7	-
	Width (cm)	(0.2-1.1)	-	(0.2-1.0)	-	0.2	0.2±0.0 (0.2-0.2)	0.2	0.2±0.1 (0.2-0.5)	-	-
Right Oviduct	Length (cm)	26.3±0.3 (17-36)	-	22.3 (17.6-32.4)	16.8	22.6±0.1 (13.0-36.0)	23.1±0.6 (18-31)	24.4±0.3 (13.36.1)	20.3±3.6 (11-31)	20.5±0.7	-
	Width (cm)	(0.2-1.1)	-	(0.2-1.0)	-	0.2	0.2±0.0 (0.2-0.2)	0.2	0.3±0.1 (0.2-0.5)	-	-
Left Horn	Length (cm)	35.2±0.6 (16-56)	-	36.3 (26.1-46.1)	28.2±0.5	38.7±0.2 (16.0-52.5)	39.2±1.7 (23.9-51)	50.3±0.3 (36.3-64)	28.8±8.5 (10-40)	36.0±2.0	-
	Width (cm)	5.98±0.1 (3.0-13.0)	-	2.3 (1.6-3.4)	-	2.6±0.0 (1.2-4.5)	1.8±0.1 (1.3-2.3)	2.7±0.0 (1.6-4.2)	-	6.8±0.5	-
Right Horn	Length (cm)	34.8±0.3 (17-55)	-	36.2 (31.0-41.5)	28.2±0.5	39.1±0.2 (21.0-53.0)	38.6±1.4 (26-49)	51.3±0.0 (37.5-64.5)	27.8±7.7 (12-41.5)	34.6±1.8	-

Organ	Measure	[23] Adult Buff. Egyptian	[24] Adult Buff. Egyptian	[9] Adult Buff. Undef. Breed	[25] Buff. Non Descript	[15] Adult Buff. Murrah	[26] Adult Buff. Murrah	[27] Adult Buff. Jaffri	[28] Adult Buff. Undef. Breed	[20] Adult Buff. Murrah	[11] Heifers Surti
Right Horn	Width (cm)	5.88±0.1 (2.9-8.9)	-	2.4 (1.0-3.3)	-	2.8±0.0 (1.4-4.0)	1.7±0.1 (1.1-2.1)	2.7±0.0 (1.9-4.4)	-	6.8±0.7	-
Uterine Body	Length (cm)	2.73±0.8 (1.4-4.8)	-	0.9 (0.7-1.5)	-	1.4±0.0 (0.5-3.0)	5.3±0.2 (4.0-7.5)	1.5±0.0 (0.5-3.5)	1.2±5.2 (0.8-2.5)	4.1±0.4	-
	Width (cm)	-	-	-	-	-	1.9±1.2 (1.4-2.5)	-	-	-	-
Cervix	Length (cm)	7.7±0.2 (4.1-11.4)	-	5.9 (3.8-9.1)	6.0±0.2	7.8±0.1 (3.0-14.0)	6.1±0.3 (4.0-9.0)	8.1±0.2 (4.0-15.0)	8.0±2.4 (2.8-11.5)	4.9±0.2	-
	No of Rings	-	-	-	3.7±0.0	3.0 (2.0-5.0)	-	3.0 (2.0-5.0)	3.0±0.7 (2.0-5.0)	3.0±0.2	-
Vagina	Length (cm)	-	-	22.1 (16.4-28.6)	-	24.4 (18.0-30.0)	-	-	17.5±7.2 (13-25)	29.5±0.6	-
Vulva	Length (cm)	-	-	10.5 (7.2-10.4)	-	12.5 (11.0-16.0)	-	-	-	-	-

Table 3. Scrotal Circumference of Buffalo (*Bubalus bubalis*) Bulls at Different Age Groups According to Different Authors

Age (in months)	**Scrotal Circumference (cm)**	**Authors**
At birth	8.7 ± 1.0 to 9.5 ± 0.0	Garcia et al. [88]; Ohashi et al. [79]
2-5	12.6 ± 0.7 to 14.0 ± 0.4	Ahmad et al. [77]; Ohashi et al. [79]; Ahmad et al. [81]
6-8	8.81 ± 0.59 to 16.3 ± 2.5	Ohashi et al. [79]; Gonçalves et al. [83]; Garcia et al. [88]; Kodagali and Doshi [89]
9-18	18.75 ± 0.93 to 21.9 ± 1.8	Ahmad et al. [77]; Ohashi et al. [79]; Gonçalves et al. [83]; Ahmad et al. [81]; Kodagali and Doshi [89]
19-24	23.92 ± 0.65 to 28.2 ± 1.6	Ohashi et al. [79]; Ahmad et al. [81]; Gonçalves et al. [83]; Kodagali and Doshi [89]
36-41	28.3 ± 0.7 to 32.7 ± 2.7	Ohashi et al. [79]; Gonçalves et al. [83]
42-47	34 ± 2.9	Vale et al. [85]
48-53	32.9±2.2 to 36 ± 3.5	Garcia [88]; Vale et al. [85]
>54	33.5 ± 1.3 to 38 ± 3.6	Garcia [88]; Vale et al. [85]

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