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Comprehensive Review on Veterinary Cocasseries in Reproduction in Male Rabbits

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ABSTRACT

The male rabbit reproductive system is crucial for ensuring reproductive efficiency and supporting both natural breeding and assisted reproductive techniques. This work offers a comprehensive overview of the anatomical, histological, and physiological features of the male rabbit reproductive system, along with an assessment of semen parameters and the factors that influence sperm quality. The organization of the reproductive organs, including the testes, epididymis, accessory sex glands, and external genitalia, demonstrates a high level of specialization needed for spermatogenesis, sperm maturation, and successful fertilization. At the histological level, the interactions within the seminiferous epithelium and along the excurrent ducts are vital for producing viable and functionally competent spermatozoa. Furthermore, the physiological regulation of reproductive function, covering puberty, hormonal control, and spermatogenic activity, is examined in relation to internal and external factors such as age, nutrition, and environmental conditions. Semen characteristics, including motility, concentration, morphology, and viability, are highlighted as key indicators of male fertility and reproductive success. This comprehensive overview provides a strong scientific foundation for understanding male rabbit reproduction and aids in improving reproductive management practices. It also lays the groundwork for developing and refining semen preservation and cryopreservation methods, which are vital in modern rabbit production and reproductive biotechnology.

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INTRODUCTION

Rabbits occupy a central place in both agricultural production and scientific research, primarily because of their remarkable reproductive capacity, rapid growth, and suitability as experimental models. Managing reproduction in this species depends on a thorough understanding of the male reproductive system's structure and function, which is essential for maintaining fertility and improving breeding outcomes. In this context, detailed knowledge of anatomical structure, histological features, and physiological processes is crucial for both natural mating and assisted reproductive technologies.

The male rabbit reproductive system exhibits a complex and highly specialized organization, including the testes, excurrent ducts, accessory sex glands, and external genitalia (Holtz and Foote 1978). These elements act together to support spermatogenesis, sperm maturation, storage, and ejaculation. On a microscopic level, the detailed architecture of the seminiferous epithelium and the dynamic cellular interactions within the testes and epididymis are vital for producing viable, functionally competent spermatozoa (Hamzah and AL-Musawi 2023). Reproductive efficiency in male rabbits is not determined by structural aspects alone; it is also influenced by physiological processes such as puberty onset, hormonal regulation, and spermatogenic activity, along with external factors like environmental conditions, nutrition, and management practices (Onuoha 2020). Assessing semen characteristics, including sperm concentration, motility, morphology, and viability, provides important indicators of reproductive performance and is commonly used in both research and field settings (El-Tarabany et al. 2015).

This work aims to deliver a comprehensive overview of the anatomy, histology, and reproductive physiology of the male rabbit, while addressing the main semen characteristics and factors influencing its quality. An integrated approach like this is essential for establishing a solid scientific foundation to improve reproductive efficiency and support the development of advanced techniques such as semen preservation and cryopreservation.

Anatomy Reminders

The reproductive system of the male rabbit includes two testes, two epididymides, two ampullae, two vas deferens, the urethra, the penis, two preputial glands, and accessory glands (Olatunji-Akioye 2020) (Fig. 1)

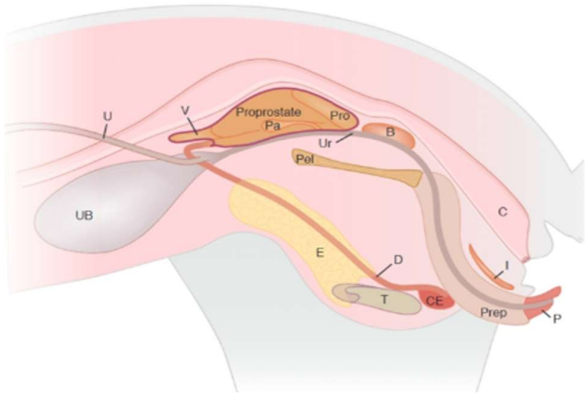

Fig. 1: Diagram of the lateral view of the reproductive tract of the buck (Meredith and Lord 2014).

The purple line refers the seminal vesicle lining which is attached to the vesicular gland, prostate, paraprostate and proprostate glands.B=bulbourethral gland; C=colon; CE=cauda epididymis; D=deferent duct; E=epididymal fat pad; I=inguinal gland; P=penis; Pa=paraprostate gland; Pel=pelvis; Prep=prepuce; Pro=prostate; T=testis; U=ureter; UB=urinary bladder; Ur=urethra; V=vesicular

Testicle

The testicle of the rabbit is a paired, elongated organ (Barrone 2001), with a rounded cranial end and a more pointed caudal end (Holtz and Foote 1978). It measures 3 to 3.5cM in length and 1 to 1.5cM in width, accounting for 1/1000th of the total body weight (Barrone 2001), approximately 2.035 ± 0.529g each (Holtz and Foote 1978). This pink organ varies in consistency depending on the subject and its physiological state, generally, they are firm and elastic (Barrone 2001) (Fig. 2).

Each testicle is contained in an individual scrotal sac in an almost horizontal position (Holtz and Foote 1978). Unlike other species, the scrotum is located cranially to the penis (Brewer 2006; Vella and Donnelly 2012). The scrotum is very thin and hairless, becoming highly visible during periods of sexual activity (Barrone 2001) (Fig. 3).

In rabbits, the position of testicles depends on several factors such as sexual activity, body position, body temperature, gastrointestinal tract filling, and the amount of abdominal fat (Capello and Lennox 2006). They descend into the scrotal sacs around the 12th week of age, and the inguinal canal does not close (Vella and Donnelly 2012). This mean that the abdominal cavity and the scrotal sacs are always in communication, allowing the testicles to move between the two (Brewer 2006) because of the highly developed cremaster muscle in rabbits (Barrone 2001).

Spermatic ducts

Epididymis

The epididymis is a tube whose length is variable according to the species (5 m in the human, 5 m in the rabbit), strongly contoured on itself and connecting the efferent ducts (at the exit of the testicle) to the vas deferens. This tube is separated into several lobules by connective partitions and has three major anatomical segments: head, body and tail (Thibault and Levasseur 2001).

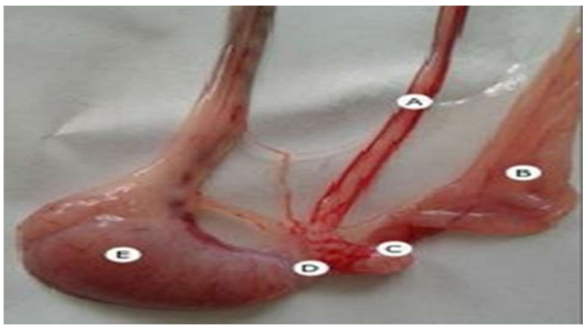

Fig. 2: A rabbit testicle (Rakhmiyati and Luthfi 2019)**.** A= vas deferens, B= Tunica vaginalis, C= epididymis, D= Ductuli efferentes, E= testis.

Fig. 3: The scrotal sacs, which contain the testicles (Carolina and Smok 2009). 1= Right testicle 2= Left testicle, 3= Foreskin, 4= Penis, 5= Anus

Head: is the largest part, it covers the cranial extremity of the testicle; the body: has a flattened shape, and the tail: is well detached, which forms a globular and mobile appendage (Barrone 2001) in the shape of a U in the rabbit (Holtz and Foote 1978) (Fig. 4).

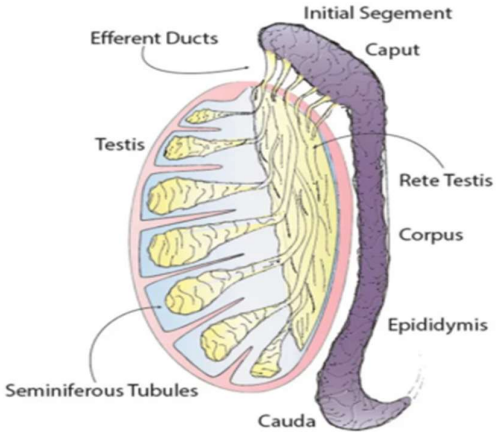

Fig. 4: Epididymis and testis anatomy (James 2020).

Different duct

It follows the epididymis, is thick, and measures 12 to 15 cm (Barrone 2001). Its first part is curved, then it becomes

straight and enters the abdominal cavity from the inguinal ring until it reaches the dorsal surface of the bladder (Leborgne and Tanguy 2013). The last part of this duct, generally called the ampulla, is about 2 cm long and has a fusiform shape, forming a circle around the ureter without its diameter changing from the rest of the canal (Campos et al. 2014).

Urethra

The urethra is the canal that runs from the bladder**.** It begins with the internal ostium of the urethra, and then it receives the endings of the spermatic ducts after a short distance, which makes its strictly urinary part very short (the urogenital sinus almost entirely forms it). In addition, it ends with the external ostium of the urethra at the free end of the penis. The role of the urethra is both the excretion of sperm and urine (Barrone, 2001).

Penis

The penis represents the copulatory organ. It has the shape of a cylinder; at its end, the diameter decreases, it measures 3 to 5 cm (Campos et al. 2014).

Unlike other mammals, the penis in rabbits is located caudally to the scrotal sacs and is characterized by the absence of the glans (Brewer 2006) and the os penis (Donnely 2004). This organ is directed obliquely backwards at rest and horizontally forwards at erection. Behind the penis, there are two preputial glands, which secrete a very odorous substance that plays a role in triggering ovulation in females (Boussit 1989).

Accessory glands

The role of genital accessory glands is to secrete the constituents of seminal plasma at the time of ejaculation (Garreau et al. 2015), their number and structure differ according to the species. The main glands are represented by the vesicular gland (glandula vesicularis), prostate (prostata), and bulbourethral gland (bulbourethralis) (Skonieczna et al. 2019) (Fig. 5).

Bulbourethral gland

A pinkish-brown glandular mass located dorsal to the pelvic urethra (covers its entire caudal part) and caudal to the prostate (Barrone 2001). Composed of two lobes separated into lobules by connective and muscular tissues, each lobe has a short excretory canal, which opens from a small hole in the dorsal surface of the urethra (Vasquez and Del Sol 2001).

Prostate

In rabbits is replaced with a glandular complex (Barrone 2001) composed of the proprostae, prostate, and two paraprostates. The proprostate and prostate are located in the dorsal region of the urethra, and the paraprostate is located dorsoventrally to the prostate (Dimitrov and Stamatova 2011). The fluid secreted by this gland represents almost half of the volume of the sperm (Skonieczna et al. 2019).

Vesicular gland

Is unpaired, large and bilobed, measuring about 25 mm. It is located dorsal to the neck of the bladder and the ampullas of the different ducts (Barrone 2001). Its extremity unites with the latter to create the ejaculatory duct (Garreau et al. 2015).

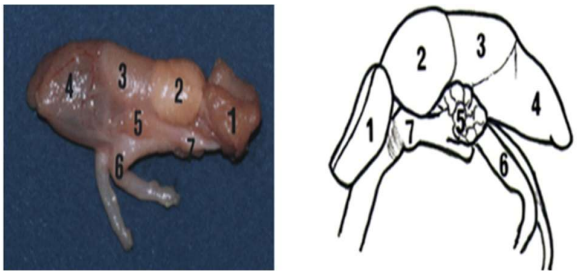

Fig. 5: Accessory glands of the male rabbit genital tract (Vasquez and Del Sol 2001). 1=Bulbourethral gland; 2=Prostate, 3=Proprostate, 4=Vesicular gland, 5=Paraprostate, 6=Vas deferens.

Histological reminders

Histology of the testis

The testicle is surrounded by a thick, resistant capsule (Varchet 1999), which is composed of dense connective tissue called tunica albuginea (Herstsh 2023), and tunica vaginalis, which are further enveloped by the layers of the spermatic cord (Christine 2024) (Fig. 6). The tunica albuginea becomes even more thicker at the epididymal cuff, sinking inside the testis to form a fibrous cone, mediastinum testis (also known as the corpus Highmori), the rete testis travelled by network of canaculi, connective septa extending from the corpus Highmori, forming the septa testis delimit 200-300 testicular lobules, with two to four extremely long and flexuous seminiferous tubes found in each lobule (Varchet 1999).

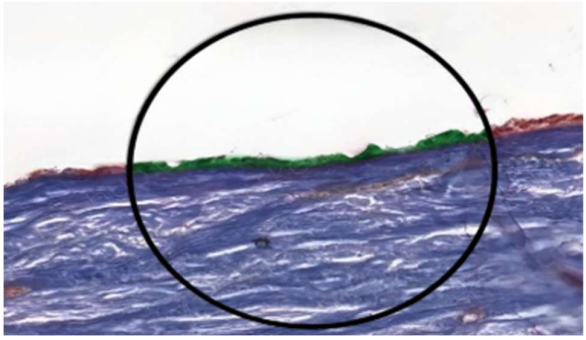

Fig. 6: Visceral layer of tunica vaginalis of testis (Christine 2024).

A cross-section of the testicle shows all the functional structures (vas deferens, epididymis, lobules, efferent ducts) and vascular elements (testicular artery, pampiniform plexus) (Fig. 7).

Scrotum and Vagina

The testicle is enveloped inside a well-developed scrotum that is located cranial to the penis and the urogenital opening **(**Capello and Lennox 2006). It consists of (Table 1).

- **Skin:** Thin, corrugated, and painted, with keratinized scaled epithelium, skin adnexae, dermis, and scattered adipocytes (Fig. 8)**.**
- **Dartos Muscular layer:** Contains smooth muscle filaments that contract in response to cold or sexual

stimulation, leading to wrinkling of the scrotal skin and bringing the testes near to the body.

- **External Spermatic Fascia (Intercrural Subcaste):** A durability of the external oblique aponeurosis.
- **Cremasteric muscle (Cremasteric layer)**: Consists of packets of cadaverous muscle, a durability of the internal oblique muscle.
- **Internal spermatic fascia (Infundibuliform layer)**: divides in the midline, extends from the transversalis fascia, and attaches to the tunica vaginalis.

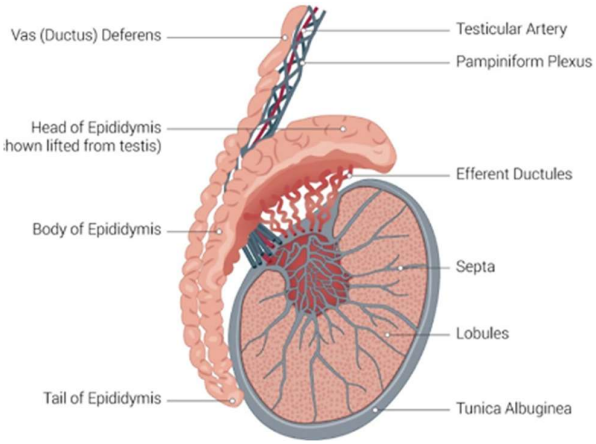

Fig. 7: Intratesticular structure (Tiwana and Leslie 2023)

Table 1: Abdominal wall and its corresponding scrotal wall layers (Abhishek 2017)

Abdominal wall	Scrotum
Skin	Skin
Scarpa's fascia	Dartos & smooth muscle
External oblique fascia	External spermatic fascia
Internal oblique muscle & aponeurosis	Cremasteric fascia & muscle
Transversus abdominis muscle & aponeurosis	Cremasteric fascia & muscle
Transversalis fascia	Internal spermatic fascia
Peritoneum	Tunica vaginalis

Parietal subcaste of tunica vaginalis: Surrounds the testes within the scrotum, the tunica vaginalis is double-layered, except posteriorly and superiorly, where it becomes continuous with the epididymis and the spermatic cord, respectively. The visceral layer of the tunica vaginalis is situated near the epididymis, testes, and vas deferens. The sinus of the epididymis is the small space found on the posterior lateral surface of the testes, between the testis and the body of the epididymis. Deep to the tunica vaginalis lies the tunica albuginea, a tough fibrous covering of the testes (Tiwana and Leslie 2023).

Testis parenchyma

Seminiferous tubules

- The length of adult seminiferous tubules is 70 cm in rabbits, the diameter is 180-250 µm, there is a lumen in the center, the wall thickness is 60-80 µm, and it is mainly composed of spermatogenic epithelium. The spermatogenic epithelium is composed of supporting cells (Sertoli cells) and 5-8 layers of spermatogenic cells. The basement membrane under the epithelium is clearly visible, and there are collagen fibers and some spindle-shaped muscle cells on the outside of the basement membrane. The contraction of muscle cells is conducive to the discharge of sperm (Wang and Zhou 2016).

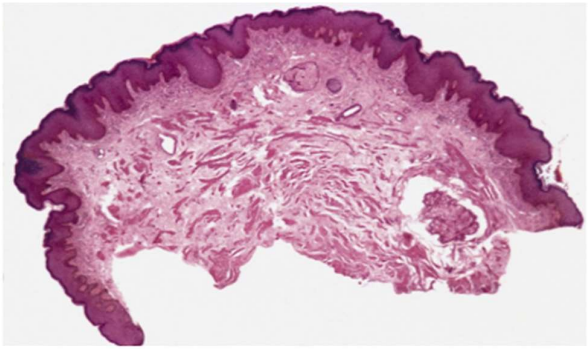

Fig. 8: Muscle bundles of the dartos are beneath the keratinized squamous epithelium de scrotum (Alcides et al. 2010).

Sertoli cells

The morphological characteristics of the rabbits' Sertoli cells can be summarized as follows**.** A branched pillar-like cell extends through all layers of the seminiferous epithelium and is closely associated with all stages of germ cell development. It has a "foot" at the limiting membrane between the apical or central end of the spermatogonia and spermatids for insertion and invagination. The nucleus is usually more or less peripherally located, and in each case, the spermatids at one stage indent the Sertoli cell membrane far enough to reach the immediate vicinity of the nucleus (Roosen-Runge 1996).

They are interconnected by continuous tight junctions that seal the tubule into two zones: the basal (near the basement membrane) and the luminal (towards the lumen) zones. Large molecules cannot pass between the basal and luminal zones - this is called the blood-testis barrier. This latter effectively isolates developing spermatogonia, spermatocytes, spermatids, and mature sperm from the blood. Differentiating sperm implant in the peripheral cytoplasmic pockets of these cells (Michelle 2003).

Germ cells

Spermatogonia, spermatocytes, and spermatids form the germinal element of the seminiferous epithelium (Abbate et al. 2010), Spermatogonia are the earliest stage of germ cell maturation and are located at the base of the tubule. During spermatogenesis, germ cells migrate from the periphery into the lumen of the tubule and undergo a strictly ordered and sequential maturation phase from spermatogonia to spermatocytes, spermatids, and mature spermatozoa. Final maturation of spermatozoa occurs in the epididymis (Treuting et al. 2017). In rabbits, spermatogenesis occurs in 48 days.

Interstitial tissue

The interstitial tissue occupies the space between the seminiferous tubules. In rabbits, like other mammals is mainly composed of Leydig cells, connective tissue, blood vessels, lymphatic vessels, various free cells such as fibroblasts, macrophages, and lymphocytes.

Leydig cells

Leydig cells of rabbits are similar to those of other mammals; they are polygonal cells with eosinophilic cytoplasm and large, round nucleus with a prominent nucleolus. They have a high lipid content and show features of steroid-secreting cells, including a large, well-developed smooth endoplasmic reticulum, large and numerous lipid droplets, and numerous mitochondria with tubulovesicular cristae. Another common finding in interstitial cells is lipofuscin, which appears as multiple round, irregular bodies representing lipid droplets accumulated in lysosomes (Aladamat and Tadi 2022).

Fetal Leydig cells and adult Leydig cells are two types of Leydig cells that have been identified (Chen et al. 2009). The fetal Leydig cells develop in utero. These cells become capable of producing testosterone (Habert and Picon 1984). Testosterone secreted by fetal Leydig cells is required for male urogenital differentiation during late gravidness (Zirkin and Papadopoulos 2018). Although fetal leydig cells express the luteinizing hormone receptor (LHR) and respond to LH stimulation (Baker and O'Shaughnessy 2001; Migrenne et al. 2001), these cells do not require LH for development. This is evident from the analysis of LHR null mice (LHRKO), in which testosterone levels during the prenatal period do not differ from wild-type control mice (Zhang et al. 2001 ; Zirkin and Papadopoulos 2018).

Different from fetal Leydig cells, adult Leydig cells develop during puberty and serve a variety of purposes, including providing the testosterone needed to initiate spermatogenesis. Different phases of the development of adult Leydig cells have been recognized and described. Leydig stem cells are undifferentiated cells with the ability to both differentiate into steroidogenic cells and self-renew endlessly. These cells produce progenitor Leydig cells, which multiply, differentiate further, and produce immature Leydig cells. The young Leydig cells produce a lot of metabolites related to testosterone. Derived from immature Leydig cells, adult Leydig cells are terminally differentiated cells. The high amounts of testosterone produced by these cells define them (Harman et al. 2001).

Epididymis histology

The epididymis consists of the efferent ducts and the epididymal duct. Between 10 and 15 efferent ducts arise from the rete testis. It consists of 3 main regional segments (head, body, and tail) (Cooper 1986). An alternate division has been suggested by Glover and Nicander (1971) based on histologic and functional criteria: initial, middle, and terminal segments. However, in this study, we will retain the most commonly used nomenclature for four regional segments : initial segment, caput, corpus, and cauda by Robaire et al. (2006) and James (2020).

The initial segment is an approximately coiled tubule with a wide diameter. In this segment are elongated cells possess high stereocilia (Wei et al. 2018).

The head (caput) receives spermatozoa and testicular fluid (Cosentino and Cockett 1985) with tall columnar epithelial cells with stereocilia that absorb this fluid and provide the sperm the optimal environment for maturation (Abbate et al. 2010).

Body (corpus): compared to the caput, it contains shorter columnar cells which continue the process of sperm maturation (Cosentino and Cockett 1985).

Tail (cauda): with shorter epithelial cells to provide the storage of sperm and maintain their viability until the ejaculation (Yamamoto and Turner 1991).

Cell Types

The epithelium of the epididymis in rabbits, like in other mammals, is specialized and varies across the different regions preliminarily mentioned to perform its functions effectively. The main cell types in the rabbit epididymis epithelium include:

Principal cells

Depending on the section examined, principal cells comprise roughly 65- 80 of the total epithelial cell population of the epididymis (Trasler et al. 1988). It has been assumed that both their structure and functions vary between the different regions**.** These differences are reflected in the appearance and association of their secretory apparatus (endoplasmic reticulum (ER), Golgi outfit and secretory grains) and endocytic apparatus (carpeted recesses, endosomes, multivesicular bodies (MVBs) and lysosomes) (Hermo and Robaire 2002). The infranuclear compartments of the principal cells are rich in rough endoplasmic reticulum, and the supranuclear compartments of these cells have multitudinous mitochondria and largely advanced Golgi complexes. The principal cells secrete carnitine, glyceryl phosphorylcholine, and sialic acid, inositol, and a variety of glycoproteins (Flickinge 1983).

Narrow, apical, and clear cells possess vacuolar H+-ATPase, which helps to secrete protons into the lumen, contributing to its acidification (Pietrement et al. 2006; Kujala et al. 2007). Apical and narrow are located only in the initial segment. Clear cells also function as endocytic cells, playing a role in the removal of proteins from the epididymal lumen and are located in the caput, corpus, and cauda regions. These cells are not located within the initial segment (James 2020). Basal cells have a close association with principal cells, as evidenced by cytoplasmic extensions that suggest basal cells regulate the function of principal cells (Pietrement et al. 2006; Kujala et al. 2007).

Finally, Halo cells are the primary immune cells within the epididymis, whereas apical cells are involved in the endocytosis of luminal components (Veri et al. 1993).

Physiology of male Rabbits Reproduction

Puberty and sexual maturity

Rabbits are known for their capacity for quick reproduction. This indicates their puberty and sexual maturity at a young age.

The age of puberty depends on several parameters, including the breed, the breeding conditions and the diet, which plays a very important role (Lebas et al. 1997; Lamothe et al. 2015). It is generally reached between the 4th and 5th month. Around the 6^{th} week, there is a quick increase in the concentrations of FSH and LH in the blood, which cause the increase in the secretion of testosterone and consequently the manifestation of puberty. The buck becomes pubescent and adopts a typically male behaviour when the testes become androgenically active, and the accessory glands begin to secrete fructose and citric acid (Skinner 1967).

Sexual maturity is defined as the moment when daily sperm production ceases to increase (Lebas et al 1997). The age of sexual maturity varies according to breeds and their body weight (Vella and Donelly 2012). In small breeds, it is earlier (4 to 5 months) than in large breeds (5 to 8 months) (Onuoha 2020). The buck shows its first signs of sexual behaviour at the age of 60-70 days; during this period, it makes its first attempts at mounting, and the first copulation can occur at 100 days (all these figures should be considered approximate) (Lebas et al. 1997).

Sperm production

Within the testes of the rabbit, spermatozoa are produced from spermatogonia and are stored inside until they are released into the epididymis along with the seminal fluid (Van Praag 2015). A healthy, well-kept, and sexually mature rabbit produces sperm throughout the year (Fodor et al. 2003).

Different daily productions of spermatozoa have been observed. According to Lebas et al. (1997), sperm production is approximately 150×10^6 to 300×10^6 spermatozoa per day. Amann and Lambiase (1967) reported a production of about $148 \pm 11 \times 10^6$ sperm per day, while Amann and Lambiase (1969) estimated it at 210×10^6 spermatozoa per day.

Spermatogenesis

Is the process by which spermatogonial germ cells develop and differentiate into mature spermatozoa. This process occurs in the seminiferous tubules of the testis over several weeks (Hermo et al. 2010). In rabbits, spermatogenesis begins between 40^e and 50^e days, and the first spermatozoa appear in the ejaculate around 110^e days (Lebas et al. 1997). The spermatogenetic cycle involves chromatic reduction (in rabbits, the chromosome number is reduced from 2n=44 to n=22) and reorganization of nuclear and cytoplasmic components (Lamothe et al. 2015) (Fig. 9).

Three main phases are distinguished:

- The mitotic phase or spermatocytogenesis: during which spermatogonia divide after replicating their DNA to give rise to primary spermatocytes.
- Meiosis: in which primary spermatocytes undergo reduction divisions, leading to the formation of secondary spermatocytes, then haploid round spermatids (n=22) (Tulsian et al. 1998).
- Spermiogenesis: here, spermatids undergo a series of modifications, without further division, leading to the release of mature spermatozoa (Baronne 2001).

Sperm maturation

After spermatogenesis, the spermatozoa produced in the seminiferous tubules are transported through the rete testis to the epididymis (Lamothe et al. 2015). Where they undergo a maturation process without acquiring capacitation, which takes place in the female genital tract. In rabbits, this journey takes 7 to 10 days.

Sperm maturation corresponds to a series of structural, biochemical and metabolic changes that enable them to fertilize the oocyte. These changes are directly linked to the metabolic functions of the epididymal epithelium and the composition of its fluids (Dadoun 1992), all under the control of androgens (Alvarino 2000).

This maturation process and the acquisition of motility occur mainly in the head and body of the epididymis (Marchiani et al. 2017). According to Lamothe et al. (2015), sperm taken directly from the seminiferous tubules are immobile. Their motility appears only at the end of the epididymal transit. Moreover, whether they are recovered from there at the beginning of the epididymis spermatozoa are not fertilizing.

Characteristics of rabbit Semen

Physical Characteristics of Semen

Volume is measured by direct reading on a graduated semen collecting tube after the elimination of the gelatinous mass (El-Desoky et al. 2017). The Quantity can vary from 0.5 to 1.5 mL (Van Praag 2015), from 0.25 to 1 mL with an average of 0.6 mL (Arencibia and Fernandez 2011).

Colour

The normal colour of rabbit semen is creamy white or milky white, and the best quality is found in the creamy white colour because it indicates a high concentration of spermatozoa (Mataveli 2008). The colour can be altered by the presence of foreign elements, such as urine, which gives a yellow colour, or red blood cells, which impart a reddish hue. These samples with abnormal colours should be discarded (Boiti et al. 2005).

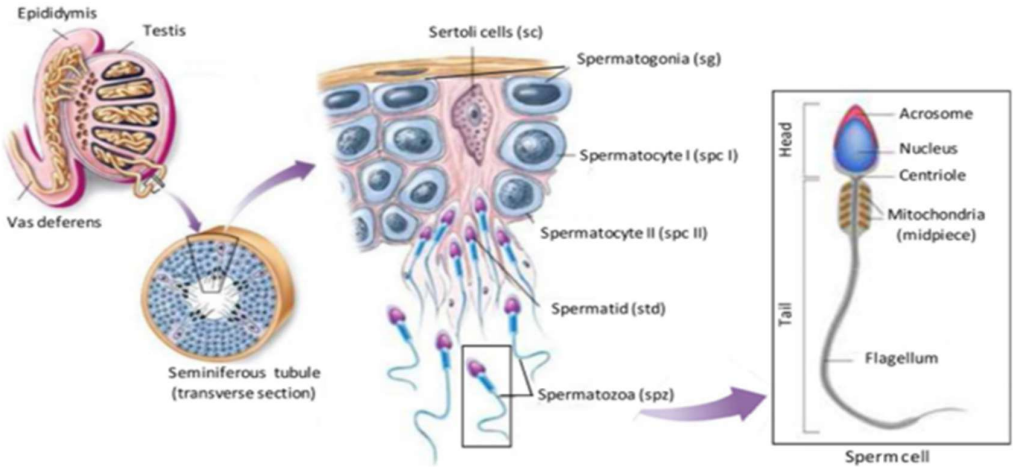

Fig. 9: Illustration of spermatogenesis (Allais and Pailhoux, 2014).

PH

The pH of rabbit semen ranges between 6.8 and 8.4 according to Alvarino (2000), and between 6.8 and 7.3 according to Arencibia and Fernandez (2011). It should be measured immediately after collection to avoid any alterations caused by metabolism (Boiti et al. 2005). To determine it, a pH meter or pH paper is used (Arencibia and Fernandez 2011).

Semen Composition

Semen represents the liquid released by the male during ejaculation. It is composed of spermatozoa, produced by testicles, and the seminal plasma, produced in different places by the accessory glands and the epididymis (El-Azim and El-kamash 2011).

Seminal fluid

Seminal plasma is the non-cellular part of sperm, represented by a fluid of heterogeneous composition, produced from the secretion of the testis, epididymis, and accessory glands.

Its role is not only to transport spermatozoa, but also to regulate their function, interact with the wall and secretions of the female genital tract, and generate energy from the constituents it contains (Rodriguez-Martinez et al. 2021), such as carbohydrates, lipids, proteins, and minerals that are essential for sperm metabolism (Onuoha 2020).

Gel fraction

Secreted by the accessory glands, this gel has a transparent consistency. Poorly soluble in extenders, which is why it is preferable to separate it from the rest of the semen before dilution (Boussit 1989).

Spermatozoa

Rabbit spermatozoon consists of a head and a cylindrical segment that includes the neck, the midpiece, and a flagellum, or tail (Fig. 10). The head: large and flattened, contains two important structures: the acrosome and the nucleus, responsible for transmitting genetic material; the middle piece is located after the neck. It measures 8.8 µm in length and is the cell's energy reservoir, due to the mitochondria it contains. The tail, with its bundles of parallel contractile fibers ending in fine fibrils forming the final segment, is responsible for the helical movement of the spermatozoa.

The entire spermatozoon measures between 50 and 60 µm, and it is enveloped by a plasma membrane whose role is to contain organelles and intracellular components (Di Iorio 2015).

Sperm Parameters

Concentration

Semen concentration is one of the key factors used to evaluate semen quality. It refers to the number of spermatozoa per unit volume (Anon, 2021). This parameter can be measured after dilution with a 1% formol-saline solution and using a hemocytometer (such as the Thomas counting chamber) (Meyer 2009). According to Lebas et al. (1997), sperm concentration in rabbits ranges from 150 - 500 × 106 sperm/mL.

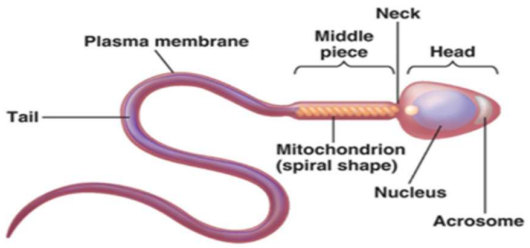

Fig. 10: Sperm cell of rabbit (Di Iorio 2015)

Viability

Viability tests assess spermatozoa plasma membrane integrity, differentiating living cells (intact membrane) from dead ones (damaged membrane) (Mocé and Graham 2008). Among the most commonly used methods in different species is the eosin nigrosin staining (Küçük 2022). In this technique, eosin stains dead spermatozoa with damaged plasma membranes, while nigrosin provides a dark background to facilitate sperm visualization. Therefore, under bright-field microscopy, viable spermatozoa appear with white or light pink heads, whereas dead ones have red or dark pink heads (Agarwal et al. 2016).

Motility

Sperm motility is one of the key factors in assessing semen characteristics, particularly for species with internal fertilisation, due to its important role in sperm transport through the female reproductive tract and penetration of the egg (Holtz and Van Look 2004).

This parameter is assessed visually as the percentage of sperm moving in a straight line. However, this visual assessment remains subjective. Therefore, it is preferable to use the CASA system for objective assessment (Chrenek et al. 2007).

Mass motility

Mass motility is a rapid and simple assessment method requiring immediate microscopic examination after semen collection (Baril et al. 1993). For evaluation, a sample of undiluted sperm is placed between a slide and a coverslip, then examined under a bright-field microscope at 10× magnification (Asibor et al. 2022). This motility is assessed using: either a 0-5 rating scale (Baril et al. 1993) or the Petitjean scale rating between 0 and 9 (Boussit 1989).

Individual motility

The analysis of individual motility (progressive motility) is recommended after diluting semen (10–40×) in an extender or pre-warmed physiological saline (Hanzen 2015). This dilution ensures each sperm cell is individually identifiable (Cabannes 2008). For examination, a drop of diluted semen is placed between a warmed slide and coverslip, then evaluated under high magnification (400×) (Hanzen 2015) using the Adrieu scale, which ranges from 0 (no movement) to 4 (rapid, small-diameter helical propulsion) (Boussit 1989).

Sperm Abnormalities

In order to judge the quality of ejaculates in rabbits, Kuzminsky et al. (1996) established criteria by examining

morphological abnormalities in spermatozoa. The results are described below:

Head abnormalities

There are two types of abnormalities: Abnormalities in shape and size which represent the minority of cases. And acrosomal abnormalities constitute the majority of cases and are classified as follows:

Acrosome bearing several 'bulges': two or more on each side (Fig. 11a). Knobbed acrosome: the external margin of the acrosome shows one or two small protrusions (Fig. 11b). Ruffled acrosome: the acrosome shows loss of turgor, and its plasma membrane is wrinkled (Fig. 11c). Retracted acrosome with swollen cords: the entire surface exhibited swollen cords and irregularly shaped protrusions (Fig. 11d). Vesiculated acrosome: many small contiguous vesicles are present on the outer surface of the front region of the acrosome (Fig. 11e). Swollen acrosome: swelling of the acrosomal cap and presence of a double head (Fig. 11f).

Tail and midpiece abnormalities

- **Tail with cytoplasmic droplets:** These droplets can occur at different levels, but they mostly occupy the midpiece (Fig. 12a, 12b). This abnormality usually leads to a tail with a marked angular deviation of the axoneme (Fig. 12a, 12b).
- Single and double bent tail: The tail presents a single bend (Fig. 12c) or multiple bends (Fig. 12d). Sometimes, the former loop is filled with cytoplasm and takes a spatula shape (Fig. 12c).
- Coiled tail: Sometimes, it takes the shape of a ring (Fig. 12e).
- Double tail: Composed of two flagella, either attached or separated (Fig 12f).

Factors influencing sperm production and quality

According to Alvarino (2000), various factors such as breed, collection frequency, feeding, and season can influence the characteristics of rabbit semen.

Breed and age

Biological characteristics of semen (volume, concentration, motility, morphological abnormalities, etc.) show significant variability both between breeds and within the same breed (Joly and Theau-Clément 2000). A study by El-Azim & El-Kamach (2011) confirms this variability, showing a highly significant breed effect on all these parameters.

Age plays a very important role as well. Research by Laurence et al. (2015) ascertained that adult males have a higher semen volume and sperm concentration than young males. Similarly, a study by Gracia et al. (2004) on the age of male rabbits in artificial insemination shows that rabbits aged 10 to 18 months have optimal sperm production and quality. Moreover, these authors suggest that rabbits can be considered physiologically immature until the age of 9 months because sperm production and quality are still low until that time.

Collection frequency

Sperm production can vary greatly from one male to another, and even from one ejaculation to another in the same individual. The quantity and quality of semen are directly influenced by both the season and the frequency of collections. An increase in this frequency leads to a decrease in sperm concentration as well as in the number of insemination doses produced per ejaculate (Joly and Theau-Clément 2000).

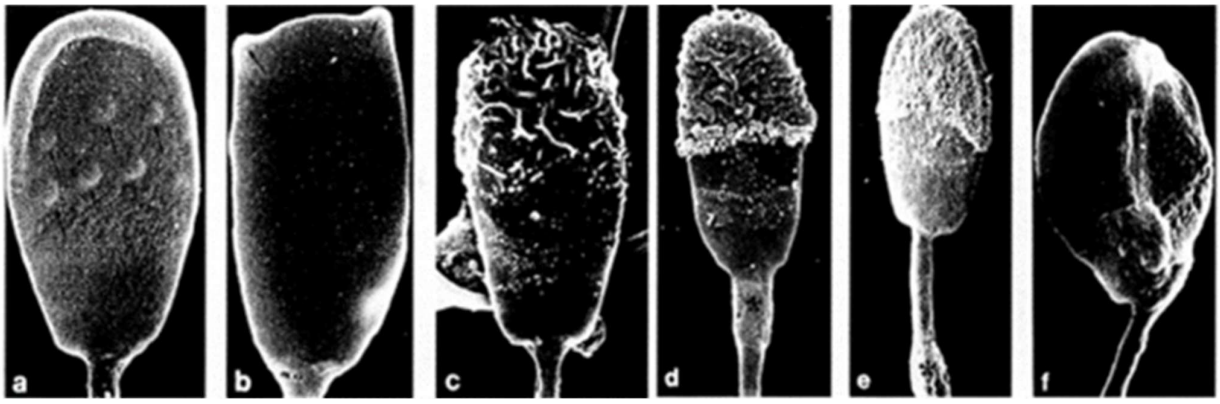

Fig. 11: Head abnormalities (Kuzminsky et al. 1996).

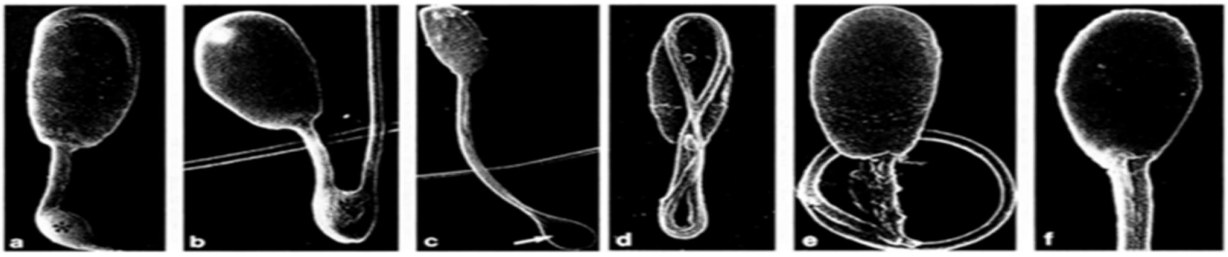

Fig. 12: Tail and midpiece abnormalities (Kuzminsky et al. 1996).

Sperm production in the testis does not appear to be affected by collection frequency. However, a high collection rate may accelerate epididymal transit and impair the process of sperm maturation within the epididymis (Benchiekh 1995).

Temperature and Seasonality

In rabbits, spermatogenesis exhibits seasonal variations influenced by photoperiod and external temperature (Alvarino 2000; Joly and Theau-Clément 2000). High temperatures affect both the quantity and quality of semen production (Marai et al. 2002). Consequently, the spermatic activity is high in spring (March to June) and low in early autumn (Alvarino 2000). Lamothe et al. (2015) found that sperm production was higher in autumn (347 and 287×10^6 total and motile sperm, respectively) than in summer (224 and 188×10^6 total and motile sperm).

Nutrition and feeding

Nutrition in male rabbits has an important impact on semen characteristics and libido. When nutritional intake is insufficient, these characteristics are affected (Joly and Theau-Clément 2000). Males fed ad libitum obtain higher volume, improved libido, and higher sperm count per ejaculate. However, sperm concentration appears to be similar to that observed in males fed with a restricted diet. Apart from a slight effect on initial pH, semen quality and pH are not affected by diet (Alvarino 2000).

Furthermore, a diet containing only 13% crude protein leads to a drop in ejaculate volume and sperm concentration (Joly and Theau-Clément 2000). This demonstrates the importance of maintaining crude protein content in male rabbits' diets above 15% (Nizza et al. 2000).

Sperm collection and conservation

Semen collection

Semen collection is the first step in assisted reproductive techniques. It can be achieved using several methods, such as artificial vagina or electroejaculation (Knox and Miller 2018).

Artificial vagina

Artificial vagina is the preferred method for semen collection (Ola 2016). Its principle is based on the sexual stimulation of the male, inducing an erection followed by ejaculation. This can be achieved by using a female in estrus, allowing the male to mount naturally, and training him to associate the collection area with sexual arousal. The device must replicate the characteristics of the natural vagina by filling it with water heated to the appropriate temperature and pressure.

This device consists of two ends: one for penis insertion and the other for the collection tube (Knox and Miller 2018). During handling, the AV is manually held under the female, with the open end pointing backward. Then it is adjusted when the male begins to mount to facilitate penetration. Once ejaculation has occurred, the device is removed (Naughton et al. 2003).

Electroejaculation

Electroejaculation is an effective semen collection method for many animal species. It involves inserting an electrical probe, adapted to the size of the animal, into its rectum, then applying a series of low-intensity electrical pulses to stimulate erection and induce ejaculation. This method does not require an artificial mounting dummy and is often combined with an artificial vagina to collect semen under optimal temperature and hygienic conditions (Knox and Miller 2018).

Semen conservation

Refrigeration

Refrigeration is a technique used for semen storage, involving lowering the temperature from +34 – 37°C to +15°C or +4°C. The cooling rate must not be too fast, due to a phenomenon known as "cold shock," which can damage spermatozoa. This phenomenon leads to a decrease in energy production, an increase in membrane permeability, circular movements, and a loss of motility.

Semen can be stored at different temperatures depending on the diluents used:

- At +4°C, it slows down sperm metabolism, conserves energy reserves, and ensures good motility after rewarming.
- Between 15–20°C, it maintains metabolism, but spermatozoa become more sensitive to toxic elements (Decuadro 2004).
- According to Boiti et al. (2005), the temperature (15 to 18°C) is generally adequate for up to 48 hours for rabbit sperm.

Cryopreservation

Cryopreservation is an effective method for preserving the structure and functionality of semen (Kamal et al. 2022). In this process, spermatozoa are packaged in straws and stored in liquid nitrogen at –196°C, which allows them to retain their fertilizing ability for several years (Knox and Miller, 2018).

Semen Extenders

Characteristics

Semen dilution is a critical step in sperm preservation and the extenders used must satisfy some very fundamental requirements: maintain sperm viability, have an adequate pH and osmotic pressure, contain energy sources (glucose, fructose, etc.), include buffer solutions and mineral ions, stabilize cell membranes, and inhibit bacterial growth (Decuadro 2004; Knox and Miller 2018; Viudes-de-Castro and Vicente 2023).

Examples of rabbit semen extenders

Tris-based extenders

Extenders containing Tris (Tris, citric acid, and fructose or glucose) form the basis of media often used for the cryopreservation of rabbit semen (Mocé and Vicente 2009). The Tris buffer plays an essential role in regulating and stabilizing pH (Gadea, 2003; Kamal et al. 2022). Roca et al. (2000) demonstrated that Tris-based extenders are effective for the dilution and preservation of rabbit semen at 15°C, and the ability of Tris-citrate-glucose to maintain sperm fertility for 48 hours at this temperature.

Egg yolk-based extenders

Egg yolk is frequently included in semen freezing extenders because of its protective role of mammalian spermatozoa against cold shock during the freezing-

thawing process. This protection is provided by low-density lipoproteins (LDL), which constitute two-thirds of the dry yolk matter (Iaffaldano et al. 2014). In the cryopreservation of rabbit semen, the EY is generally integrated into diluents at concentrations ranging from 10 to 20% (Mocé and Vicente 2009), acting as a non-permeable cryoprotectant that prevents the degradation of the plasma membrane (Kamal et al. 2022).

Milk-based extenders

Extenders based on skimmed milk (at a final concentration of 8 to 10%) have also been used to preserve rabbit semen, although their use is less common than that of egg yolk (Mocé and Vicente 2009). A comparative study by Suarez et al. (2020) concluded that diluents composed of skim milk or caseinates, as well as sugars, more effectively protect rabbit semen stored by refrigeration.

Commercial extenders

Various commercial diluents are used in artificial insemination of rabbits, the most commonly used including: Ringer-Locke, Salisbury, Tris buffer, Spermasol, Lepus® (MEDI Chimica, Italy), and Dilap 2000® (Duranti et al. 1993). However, the exact composition of many extenders remains unknown due to commercial interests (Viudes-de-Castro and Vicente 2023).

Several comparative studies have evaluated the effectiveness of these extenders. For example, Carluccio et al. (2004) demonstrated that INRA 96® maintains significantly higher concentrations of viable and motile spermatozoa, stored either in refrigeration or at 38°C, compared to other solutions such as Lepus® and Verdünnungsmischung M III. Also, Bresciani et al. (2016) recommended the use of Formula V® for rabbit artificial insemination programs.

Conclusions

The present work offers a detailed overview of the male rabbit reproductive system, combining its anatomical, histological, and physiological features with characteristics of semen and the factors that influence its quality. The organization of reproductive organs, from the testes to the accessory glands, shows a high level of specialization that supports effective spermatogenesis, sperm maturation, and successful fertilization. Histological features, especially within the seminiferous tubules and epididymis, emphasize the coordinated cellular interactions necessary for sperm production and functional viability.

Furthermore, the physiological processes involved in puberty, spermatogenesis, and sperm maturation illustrate the complex regulation of male fertility, influenced by both internal and external factors, including age, nutrition, environmental conditions, and management practices. Evaluating semen characteristics and sperm parameters remains a key method for assessing reproductive performance and identifying potential issues, and determining the cause is an essential step to finding the solution and acting accordingly.

Overall, a comprehensive understanding of the structural and functional dynamics of the male rabbit reproductive system is crucial for enhancing reproductive efficiency and optimizing assisted reproductive techniques. This knowledge provides an essential foundation for the development and improvement of semen preservation methods, including refrigeration and cryopreservation, which are important in modern rabbit production and reproductive biotechnology.

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