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MORPHOMETRIC AND REPRODUCTIVE
CHARACTERIZATION OF THE LOCAL
POPULATION RABBIT: Case of the Tiaret region

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**To my mother;
Wise in counsel, tender in judgment,
And in all charity,
Strengthful in faith and purpose;
I dedicate, with love, this dissertation.**

**To my father;
BERROUAGUIA HADJ,
With reverence.**

**Actually, Mom and Dad don't speak English, so if no one tell them about
this, they'll never know.**

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PUBLICATION

Sperm quality in response to age in local rabbits reared in semi-arid environment (Tiaret, Algeria)

ABSTRACT

The aim of this study was to assess a morphometric and reproductive characterization of local rabbit bucks in the region of Tiaret. The study was conducted at the experimental farm of Ibn Khaldoun university of Tiaret. A total of 20 rabbit bucks of the local Algerian population (5-11 months of age) weighting between 3010g and 4540g were collected randomly and exposed to an extensive rhythm of collection. The average value of libido was $25,17 \pm 20,94$ seconds (sec.). The ejaculate volume was $1,48 \pm 0,33$ ml and the pH $7,67 \pm 0,36$ for bucks of 11 months of age. The analyses of semen show no significant for mass and individual motility ($6,84 \pm 1,70$ and $2,96 \pm 1,04$ respectively). The rate of vitality was $61,18 \pm 18$. However, the age of bucks significantly affected the concentration and abnormal spermatozoa ($p < 0,05$). Most of semen parameters were influenced by the age and rabbit bucks of the Algerian local population seems desirable for reproduction in compare with other strains. For the external morphometry; weight, sizes related to general aspect, head, trunk, limbs were measured and fur coloration was appraised. The results of measured parameters allow the population to be classified in the category of small rabbit breeds.

Key words: *Local rabbit, reproduction, morphometry, sperm*

Résumé

L'objectif de cette étude était la caractérisation morpho-métrique et reproductive des lapins locaux dans la région de Tiaret. L'étude a été menée à la ferme expérimentale de l'université Ibn Khaldoun de Tiaret. Un total de 20 lapins de la population algérienne locale (5-11 mois) pesant entre 3010g et 4540g ont été exposés à un rythme extensif de récolte. La valeur moyenne de la libido était de $25,17 \pm 20,94$ secondes. Le volume de l'éjaculat était de $1,48 \pm 0,33$ ml et le pH de $7,67 \pm 0,36$ pour les mâles de 11 mois. Les analyses du sperme n'ont montré aucune différence significative pour la mobilité massale et la motilité individuelle à savoir $6,84 \pm 1,70$ et $2,96 \pm 1,04$ respectivement. Le taux de vitalité était de $61,18 \pm 18$. Dans cette étude, l'âge des mâles a affecté significativement la concentration et le taux des spermatozoïdes anormaux ($p < 0,05$). Dans cette étude, la plupart des paramètres de la semence ont été influencés par l'âge et les mâles de la population locale algérienne semblent avoir les qualités nécessaires pour une bonne reproduction. Pour la morphométrie externe ; le poids, les tailles liées à l'aspect général, la tête, le tronc, les membres ont été mesurés et la coloration de la fourrure a été évaluée. Les résultats des paramètres mesurés permettent de classer la population dans la catégorie des races de petits lapins.

Mots clés : Lapin local, reproduction, morphométrie, sperme

المخلص

كان الهدف من هذه الدراسة تقييم الخصائص الشكلية والتناسلية للأرانب المحلية في منطقة تيارت. أجريت الدراسة في المزرعة التجريبية التابعة لجامعة ابن خلدون بتيارت. تم جمع 20 من الأرانب المحلية والتي تتراوح أعمارهم بين (5-11 شهرًا) بوزن 3010 غم حتى 4540 غم بشكل عشوائي. لقد تتراوح متوسط قيمة الرغبة الجنسية بين 20.94 ± 25.17 ثانية. بينما تتراوح حجم السائل المنوي 0.33 ± 1.48 مل ودرجة الحموضة 0.36 ± 7.67 للأرانب ذات 11 شهرًا. أظهرت تحليلات السائل المنوي عدم وجود معنوية لحركة الحيوانات المنوية الفردية والكتلية إذ قدرت ب (1.70 ± 6.84 و 1.04 ± 2.96 على التوالي) و كان معدل الحيوية 18 ± 61.18 . إلا أن عمر الارانب أثر بشكل معنوي على التركيز و نسبة الحيوانات المنوية غير طبيعية ($p < 0.05$)

في هذه الدراسة ، تأثرت معظم معايير السائل المنوي بالعمر وتبدو الأرانب المحلية مستحسنة للتلقيح مقارنة بالسلالات الأخرى.

فيما يخص قياسات الشكل الخارجي. تم قياس وزن الأرانب والمقاسات المتعلقة بالجانب العام والرأس والجذع والأطراف وتقييم لون الفراء. تسمح نتائج المقاسات بتصنيف هذه الأرانب في فئة سلالات الأرانب الصغيرة.

الكلمات الدالة: الأرانب المحلية ، قياس المرفولوجيا ، التكاثر، المنى

ABBREVIATIONS KEYS

AS: Abnormal spermatozoa

BL: Body length

CC: Chest circumference

CP: Crude Protein

DE: Distance between eyes

EL : Ear length

EW : Ear width

FFC: French Federation of Cuniculiculture

FSH: Follicle stimulating hormone

GM: Geometric morphometry

GC: Geometric conformation

HC: Heat stress

HL : Length of head

i.e.: *id est*, which means “That is”

IM: Individual motility

LBL: Lower back length

LBW: Lower back width

LH: Luteinizing hormone

LP : Leg length

LQ : Tail length

LW: Live weight

MM: Mass motility

SD: Standard deviation

TF : Turn of the forelimb

TL : Total length

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GENERAL INTRODUCTION

The diversity of domestic animal populations, resulting from human-led selection in various farming systems, is declining rapidly as a result of the intensification of animal husbandry in the second half of the 20th century. The extreme specialization of certain domestic breeds and their worldwide dissemination have in fact been achieved to the detriment of less productive local breeds, and therefore of biodiversity (Larrivière and Leroy, 2008).

The spread of domestic rabbit farming outside of Europe is a historically recent phenomenon that has been around two or three centuries at most, and most often less than 100 years ago. The establishment of the wild rabbit was a "success" where the climate was close to that of the region of origin of the rabbit but above all where the ecological niche was free, where there were no predators. From its geographical origins, the rabbit has an adaptation to the Mediterranean climate with hot and dry summers and winters which can be cold and to the variability of fodder resources in the Mediterranean zone: strong in spring, modest in summer then increasingly rare in autumn (Lebas, 2004).

The local Algerian rabbit population has only been characterized phenotypically, based on qualitative characters and zoo-technical performance. Indeed, only one race called "the Kabyle" was described for Algeria by Khalil and Baselga (2002). The introduction of different European breeds to improve rabbit production have created an anarchic mixture with loss of local breeds (Berchiche *et al.*, 1999).

The strategy for the development of the rabbit species in Algeria is currently based on the valorization of rabbits from the local population (Gasem and Bolet, 2005). This valuation must first of all include its characterization on the morphological level and the knowledge of its biological and zootechnical aptitudes in order to guide its use (Nezar, 2007).

The aim of this work is to assess reproductive and morphometric characteristics of local Algerian rabbit bucks in the Tiaret region, and improve the state of knowledge on the potential of our animal heritage.

PART ONE:

LITERATURE

REVIEW

CHAPTER I

RABBIT; BREED AND MORPHOLOGY

I.1. Definition and origins

Rabbit is a lagomorphic mammal of the *Leporidae* family. The European rabbit (*Oryctolagus cuniculus*) which has 38 important breeds and 77 varieties, is the most famous rabbit and the only species of the genus *Oryctolagus*, It is common in Europe but it was imported later in Australia where it proliferated. Until the Middle Ages, it was often raised in semi-captivity, in large enclosed spaces called warrens, but its domesticated form, raised in hutches, is common all over the world. Indeed, it is the strain species of all domestic rabbits, with many breeds and varieties. (Bharathy *et al.*, 2021).

I.1.1. Rabbit species in Algeria:

According to Berchiche and Kadi (2002), there was no study on the local rabbit before 1990, but rabbit farming has existed for a very long time in Algeria (Ait Tahar and Fettal, 1990). It seems that the North African rabbit was introduced by the Romans across the Iberian Peninsula half a century BC, and seems to have been maintained there as small rural herds (Barkok, 1990). In the 19th century, the colonization and the arrival of populations of European origin traditionally consuming rabbits, more recently, led to the development of rational units in the Maghreb, but this rational sector only appeared in Algeria at the beginning of 80's (Colin and Lebas, 1995).

The rabbit species present in Algeria are represented by the taxonomic family of *Leporidae*, which includes domestic rabbits (*Oryctolagus cuniculus domesticus*) and hares (*Lepus capensis*) or "the brown hare". Phenotypic resulting from untimely and sometimes deliberate crosses (search for performance characteristics) with foreign breeds introduced in Algeria, during the 1970s, as part of some rural development projects (the White New Zealand, the Fauve of Bourgogne, the Giant of Flandres, the Californien and even the Giant of Spain). This process was aggravated by the introduction, between 1985 and 1989, of selected breeders (hybrids like Hyla and Hyplus), intended for intensive breeding (Berchiche and Kadi, 2002; Ferrah *et al.*, 2003; Othmani and Benazzoug, 2005; Djellal, Mouhous and Kadi, 2006). According to Berchiche and Kadi (2002), and Djellal, Mouhous and Kadi (2006), the result of these random introductions was an anarchic mixture and the loss of the native rabbit in certain regions (La Kabylie). (Nezar, 2007).

I.1.2. Rabbit breeds and Classification criteria:

I.1.2.1. Breeds concept:

According to Lebas (2002), the best of the variable definitions of race may be that of Quittets: “Race is, within a species, a collection of individuals having in common a certain number of morphological and physiological characters that they perpetuate when they reproduce with each other” (Lebas, 2002).

The different selections made over time have served to fix the useful or appreciated characters, and to eliminate the undesirable aspects, to arrive at the formation of the races, which should not however be regarded as static but always in process of evolution and of selection (Gianinetti, 1991).

I.1.2.2. Classification criteria:

Rabbit breeds are often grouped, for convenience, based on adult weight or adult height, the majority of selections regarding size and body morphology have separated these breeds into four types of categories: Giant (heavy), medium, small (light) and dwarf. Heavy breeds are characterized by an adult weight of more than 5 kg.

The largest breed is the Flanders Giant (7 to 8 kg) followed by the French Aries and the French Butterfly Giant. Medium breeds, ranging in adult weight from 3.5 to 4.5 kg, are the basis of the breeds used for intensive meat production in Europe. We can cite as examples the Californian Himalayan, the Fauve de Bourgogne or the New Zealander White, the breed most used for commercial production. Among the light breeds, whose adult weight is between 2.5 and 3 kg, are the Russian, the Small Chinchilla or the English Silver. Finally, dwarf breeds, with an adult weight of around 1 kg, are often used to produce pet rabbits. These breeds include the colored dwarf rabbits or the Polish rabbit (Chantry Darmon, 2005).

The origin of the breed is a major determinant of its adult size, the latter is also related to the zootechnical characteristics of the breed such as: precocity, fertility, growth rate and age of maturity and therefore with the zootechnical orientation of the breed (Lebas, 2002).

I.1.3. Rabbit local population and Livestock:

I.1.3.1. Population:

For the geneticist, a population is a set of animals effectively reproducing among themselves (De Rochambeau, 1990). Most rabbits used for commercial meat production most often belong to populations of animals that may resemble a particular breed (matter of appearance only, without meeting the criteria of origin and standard of the breed.), or look like no race. These are "common" rabbits resulting from various unplanned crosses (farm breeding) or belonging to local populations (Lebas, 2002).

I.1.3.2. Local population

It is defined as being a geographic population (De Rochambeau, 1990). Third world countries may have local populations, for example, the Baladi rabbit from Sudan or Egypt, the Maltese from Tunisia, the Creole rabbit from Guadeloupe (Lebas, 2002).

The functioning of these populations is characterized by human action who defines a standard and selects for compliance with that standard; for example, the Fauve de Bourgogne comes from the fawn rabbits of the local population of Burgundy (population French farm geography) selected with patience (De Rochambeau, 1990; Bolet, 2000). Breeds can, however, constitute genetic pools with interesting potential for the improvement of these local populations (Lebas, 2002).

I.1.4. Characterization of rabbit livestock in Algeria:

According to Colin and Lebas (1995), Algeria is among the countries where rabbit farming is quantitatively quite important but which remains very traditional and almost exclusively food and where the production of rabbit is intended almost exclusively for home consumption or for supply of meat to the farmer's immediate environment (family, neighborhood, etc.). These traditional farms live practically in autarky and because of their absence of contact with other economic agents, are generally underestimated during official censuses, hence an underestimation of the volume of rabbit farming in Algeria, this rabbit culture is that of most of the countries of North Africa: Egypt, Morocco, Tunisia... etc. (Colin and Lebas, 1995).

I.2. Morphometrics and Rabbit morphology:

According Needham (1950) morphometry is the measure of the shape of an object, including both its size and its conformation, according to the relationship: "Shape = Size + Conformation" (Needham, 1950).

The morphological characteristics by which a purebred rabbit is described in a standard are six: the first three are similar for all racial descriptions and relate to general appearance, mass and size, coat. Then come three positions which take into account the characteristics specific to each breed and which make its originality, it can be about the color, the drawing, the shape and the length of the ears and the head (Boucher and Nouaille, 2002).

I.2.1. Type and general aspect:

It is the general description of the animal's physique, it is used to indicate the body conformation of the rabbit or the size of a part of its body such as "head type". The coordinates of the type of the animal are: general appearance and size and by extrapolation, weight (FFC, 2000).

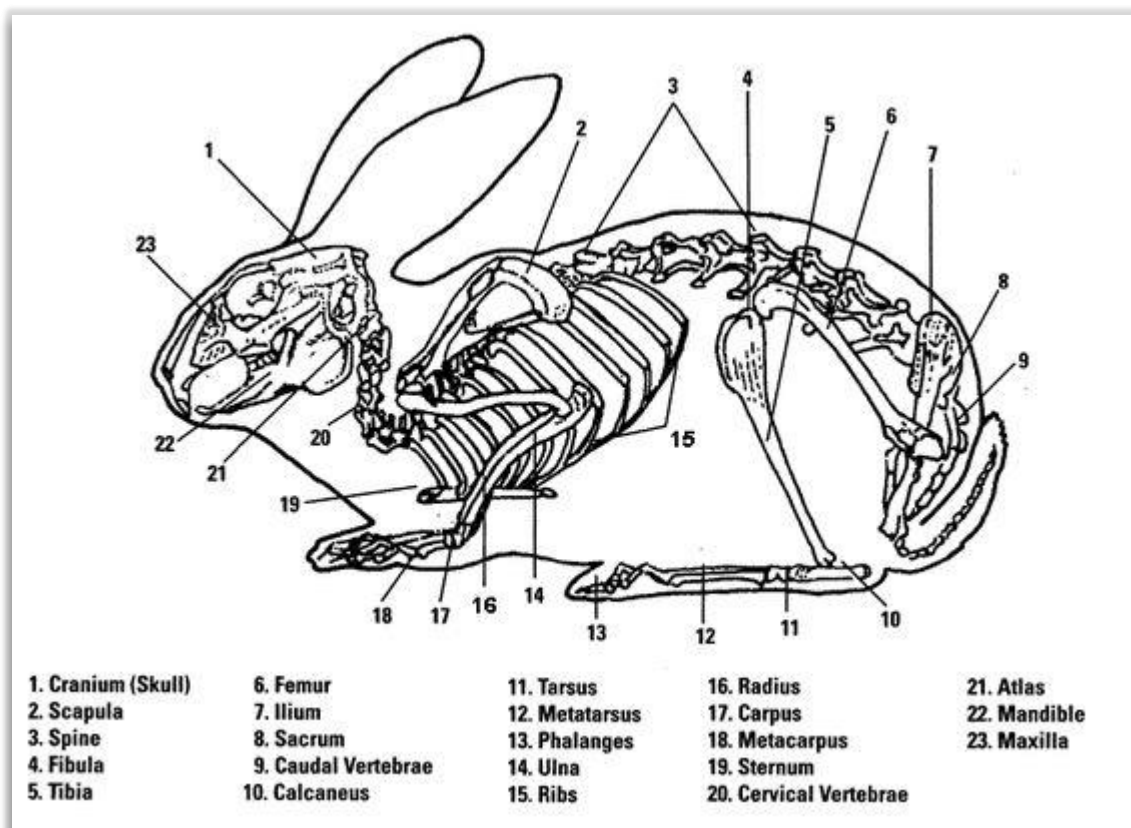


Figure 1. External parts and skeleton of the rabbit. (Barone *et al.*, 1973)

I.2.1.1. Head: At the level of the animal's head, it can be distinguished:

- **The ear:** on the lateral border of the external auricle, a lateral and medial superficial auricular veins can be observed. It is possible to place a venous catheter on these atrial veins (as we will see in the clinical applications described below) (Barone *et al.*, 1973).

A superficial atrial artery is located in the middle of the external atrial flap. Of arterial blood samples can be made at this level.

- **The eye:** protected by three envelopes, namely, the upper eyelid, the lower eyelid and the third eyelid. The latter reclines in the horizontal direction in the rabbit. The rabbit presented with physiological exophthalmos.

Behind the eye is a venous sinus, which should be taken special care of during enucleation.

- **The nostrils:** covered with numerous vibrissae, which play an important role in the perception of the environment.
- **The jugular groove:** along which runs the external jugular vein, visible when compressing at the base of the neck; blood tests are possible at this level (as we will see in the clinical applications described below). These blood tests are more difficult to perform because of the less obvious contention of this region.

I.2.1.2. Trunk (Barone *et al.*, 1973)

- Dorsal line which extends from the neck to the croup where the spine continues, supported by twelve thoracic vertebrae and then seven lumbar vertebrae. Its trajectory is regular, more or less curved, without any sagging or protrusion.

Seen from above, this dorsal line has an almost identical width over its entire extent with, however, a thickening of the muscle masses at the level of the saddle. (Figure 1)

- Shoulders well developed and tight to the body, which makes it difficult to perceive the movement of the scapulae, the pits of which are furnished with muscles.
- The pectoral region is ample and sufficiently let down, which does not let perceive of sternal projection. The ribs are correctly arched, they stretch laterally and from front to back to give a somewhat curved configuration to the thorax,
- The abdomen is not distended and well supported.

- The croup is supported by four fused sacral vertebrae and pelvic bones to which are added the first coccygeal vertebrae. It is clearly rounded without any bony protrusion and extends laterally through the thighs (FFC, 2000; Lebas, 2002).

According to Barone *et al.* (1973); the trunk of the animal can be divided into four main parts:

- The neckline;
- The thorax; whose volume is relatively small compared to the size of the animal. This characteristic is one of the explanations for the sensitivity of the respiratory system of rabbits to various pathological conditions.
- The abdomen; rabbit has a very long lumbar region which predisposes it to trauma. Indeed, a wrong movement can lead to the formation of fractures of the lumbar vertebrae which can be fatal for the animal, hence the interest of a good restraint during the clinical examination.
- The Pelvis.

I.2.1.3. Limbs

The upper extremities defined dorso ventrally by the following regions, i.e., shoulder, arm, forearm, carpus, fingers. On the forearm level, when performing a compression, the cephalic vein can be observed and catheterized (Figure 1).

Pelvic members, defined dorso ventrally by the following areas, namely region gluteal, thigh, stifle, leg tarsus fingers.

Rabbits have the particularity of being plantigrade and having the feet and palms of the hands covered with hair, which reduces the appearance of pododermatitis in this species.

However, there are "sore leg" in farmed rabbit due to an infection of staphylococcus.

I.2.1.4. Tail:

It originates at the base of the rump and is placed on the body of the rabbit. It is sufficiently long and includes the last coccygeal vertebrae, also called caudal (FFC, 2000; Lebas, 2002).

I.2.2. Size:

This is the criterion adopted for breeds and varieties, it depends on the elongation of the skeleton of the animal allowing rabbits to be classified into giant, medium, small and dwarf breeds (FFC, 2000). The body length of the animal and the number of vertebrae it has are characteristics of high transmissibility, they pass easily from parents to descendants and are absolutely not influenced by the environment. They appear from birth, although they are not very evident at this time (Gianinetti, 1991).

I.2.3. Weight:

This is the average specific weight achieved by adult rabbits of a given breed. The classification according to weight differentiates four types of rabbits: heavy breeds (between 5 and 7 kg), medium breeds (from 3 to 5 kg), light breeds (from 2 to 3 kg) and dwarf breeds (from 900 g to 2 kg) according to Lebas (2010).

CHAPTER II

REPRODUCTIVE PHYSIOLOGY IN RABBIT'S BUCKS

II.1. Anatomy of the reproductive system

II.1.1. External reproductive system and sexing:

The reproductive system of rabbit buck (Figure 2) consists of the testes (2) which weighed over 6 grams in some breeds (Herbert *et al.*, 2005), epididymis (2), ampoules (2), Vas de-ferens (2), urethra, penis, preputial glands (2), accessory glands, a well-developed scrotum located adjacent to the penis and the urogenital opening (Campos *et al.*, 2014) (Capello and Lennox, 2006).

II.1.1.1. Scrotum

The testicles are housed in the scrotum. The scrotum is formed by the tunica vaginalis, tunica dartos and cremaster muscle and said to have few hairs (Donnelly, 2004). Its main function is to keep the testicles away from the abdominal cavity so that the right testicular temperature is maintained between 0.5°C and 4°C below body temperature as required for normal spermatogenesis (Alvariño, 1993). The scrotum and abdomen have communication through the inguinal ring which conveys the excretory duct (vas deferens) that comes from the epididymis.

II.1.1.2. Penis

Although the gross anatomy between rabbits and other animals is different, histological structural elements are similar in mammals, but with special characteristics for each species (Banks, 1992). An unusual feature of the rabbit is the absence of glans in the penis (Brewer, 2006) but the body of the penis is cylindrical, 40 - 50 mm long and the diameter decreases at its end. During rest from sex, the penis lies in the foreskin located ventrally to the anus (Brewer, 2006) and caudally to the testicles (Capello and Lennox, 2006).

II.1.1.3. Epididymis

The epididymis is the place where sperm produced are stored before being released during ejaculation. Its functional part consists of a single duct. The epididymis originates in the efferent ducts; highly curled over the head, body and tail and connects straight to the vas deferens. It is noted by some authors that the tail of the epididymis is in the shape of a U (Holtz and Fouts., 1978). The rabbit is one of the species in which sperm stored in the cauda epididymis exhibit vigorous motility even in their own fluid (Turner and Reich., 1985).

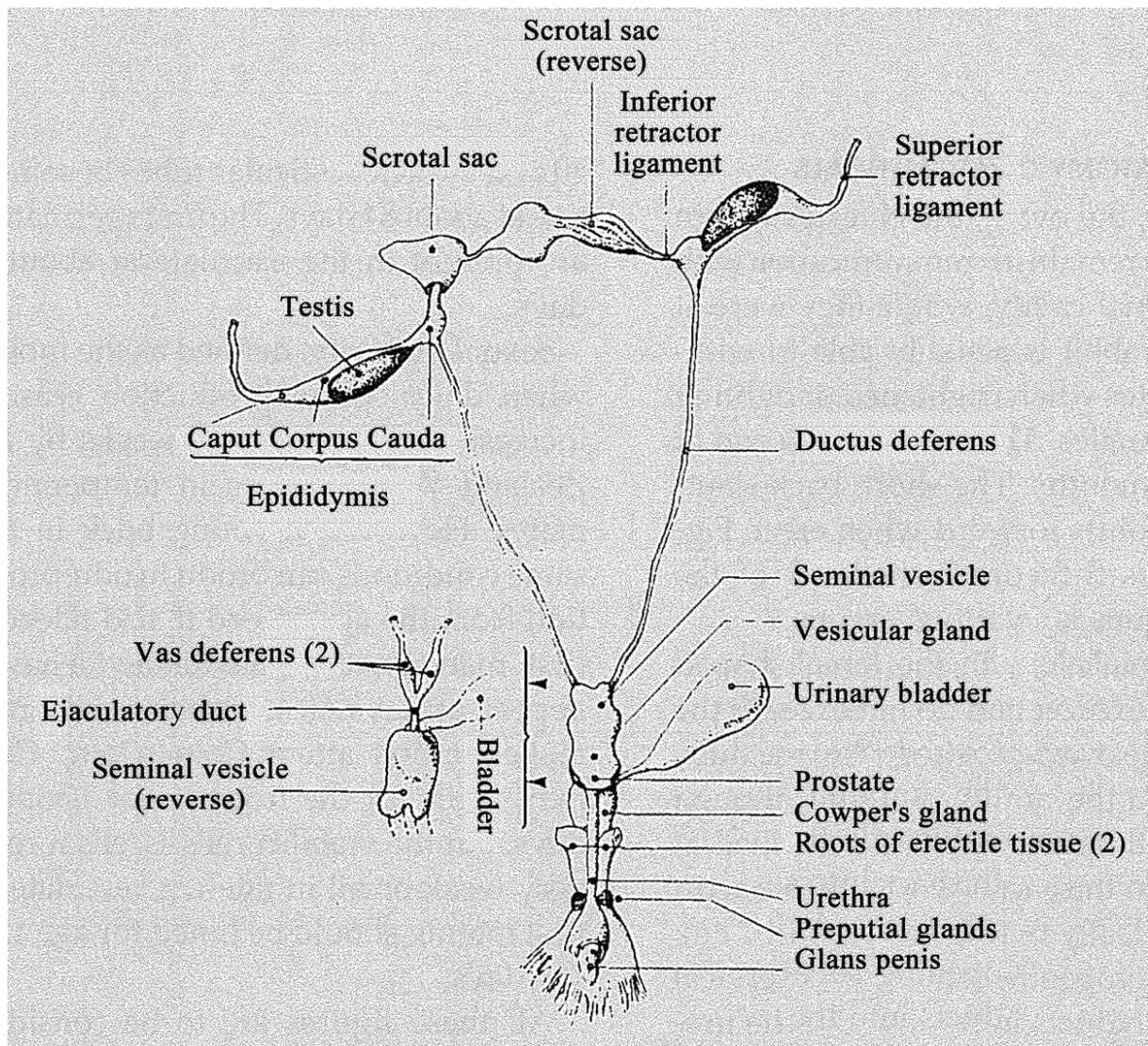


Figure 2. Genital anatomy of the male rabbit. (Lebas *et al.*, 1997)

II.1.1.4. Vas Deferens

The vas deferens extends dorsal-cranial to the body of the epididymis through the inguinal canal and enters in the abdominal cavity. The final portion of the vas deferens forms a loop around the ureter and at this point becomes fusi-form. Although the thickness of the diameter does not differ from the rest of the vas deferens, this segment is generally called ampulla (ampulla vas deferens) (Holtz and Fooks., 1978).

II.1.1.5. Urethra

The urethra is a large canal that leads through the penile organ to the outside of the body. It is the connection point at which the two vas deferens ducts converge from the left and right sides of the body to connect at its upper end; very near to where the urinary bladder opens into the urethra (Campos *et al.*, 2014).

II.1.1.6. Testes

The testis of the buck is oval-shaped. It is the main source of testosterone in rabbits (Castro *et al.*, 2002) which is the main androgen produced during sexual maturation (Chubb *et al.*, 1978). They are positioned cranially to the penis according to Capello and Lennox (2006) ; Brewer (2006), located in the scrotum, each one on one side of the inguinal line and positioned almost horizontally (Holtz and Fouts., 1978).

After birth, the testes develop less quickly than the rest of the body. From the age of five weeks, they begin to grow very rapidly. The rabbit's testicles descend at about two months and they are said to be similar to those of cats but can move freely from the scrotum to the abdomen through an opening in the inguinal canal Brewer (2006). The testicles continue to grow and increase sperm production until six (6) months of age (Morton, 2006).

The position of the testicles depends on many factors including body position, body temperature, reproductive activity, repletion of the gastrointestinal tract, amount of abdominal fat (Capello and Lennox., 2006) and stress (Richardson, 2003). During periods of sexual inactivity or stress, the testicles return to the abdominal cavity through the inguinal ring and may go down again by the action of the cremaster muscle (Capello and Lennox., 2006) Alvariano (1993).

According to Fraser (1988), the appearance and testis weight depend on the location. For example testes located in the scrotum are heavier, firm in texture and red in colour. Abdominal testes are light, reddish-brown and limp.

Although their essential function is the maintenance of normal spermatogenesis, serum testosterone above the baseline level do not appear to influence the efficiency of spermatogenesis (Castro *et al.*, 2002).

II.1.1.7. Accessory Glands

The accessory sex glands are complex (Figure 3). They secrete many compounds found in the semen of other mammals such as fructose, citric acid, glycerylphosphorylcholine, and minerals according to Holtz and Foote (1978). Secretion of catalase (Foote and Hare, 2000) is uniquely high in rabbit semen. The accessory sex glands respond differentially to androgens and estrogens (Foote *et al.*, 1977) and the weights of this organ are a bio-indicator of circulating steroid hormone levels. Accessory glands in the rabbit develop less quickly than the rest of the body just like the testes but at a more even rate and are less precocious. The glands of the rabbit's reproductive tract differ in number, location, size and proportion among other aspects like those in other mammals. This set of glands consists of a vesicular gland, bulbourethral gland and prostate gland (Dimitrov and Stamatova, 2011) and Vasquez (2002) affirm that the prostate consists of three lobes: Proprostate, Prostate and Paraprostate. According to (Hafez, 1995), they contribute to the greater part of the volume of ejaculate. Each part of the gland plays a specific role in reproduction (Dimitrov 2010).

II.1.1.8. Prostate Gland

The prostate gland is yellowish-white in colour and is located caudally to the vesicular gland and found in between the Pro prostate and bulbourethral glands. It shares the same connective tissue capsule as the pro prostate; only a small layer of tissue separates these two glands. The paraprostate glands are small and were named as such because they are located on both sides of the prostate (Dimitrov and Stamatova, 2011). They have an irregular embossed surface and are hammer-shaped (Vasquez *et al.*, 2002).

II.1.1.9. Bulbourethral Gland

The bulbourethral gland of the rabbit is a small mass of glandular tissue that is surrounded by a capsule and widely covered by skeletal bulb glandular muscle that separates it into lobules. This gland originates in the urethral wall, as distinct from other species. It is fairly small in rabbits but relatively larger than that of a man (Vásquez and Del Sol, 2001).

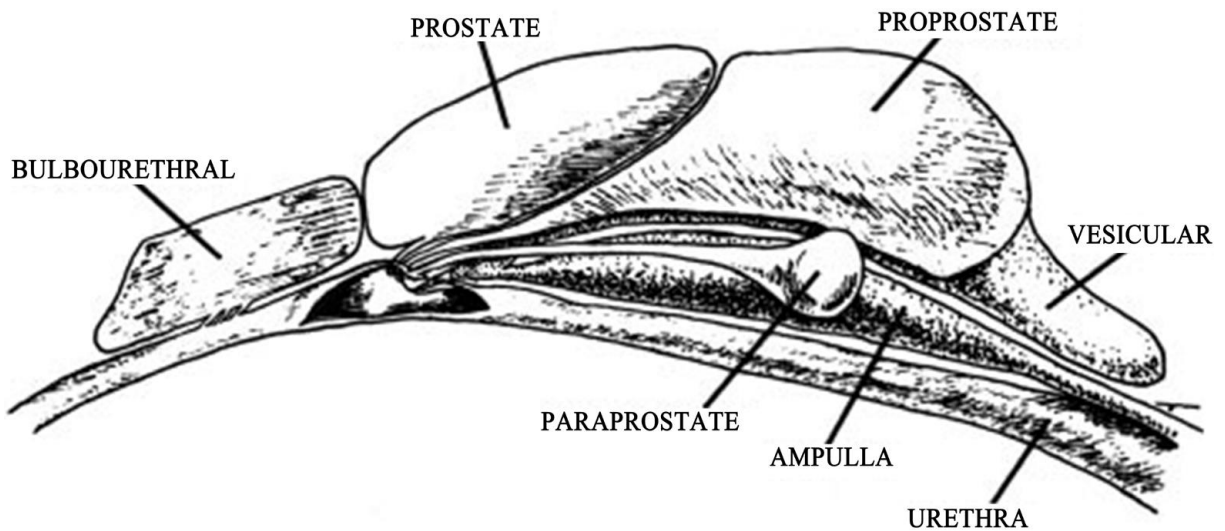


Figure 3. Midsagittal section through the urethra and the accessory sex glands of the domestic male rabbit. With the exception of the ejaculatory duct (Holtz and Foote., 1978) (all ducts are paired).

II.2. Reproductive Physiology

The onset of reproductive cycle marks the attainment of puberty depending on the level of feeding and breed. This is about 4 - 5 months in light breeds (Fielding, 1991). At puberty, the reproductive organs are fully developed. The testicles are housed in the scrotum.

The male sex cells are formed in the tiny seminiferous tubules which are basically two-ended loops with two ends opening into the rete testis, each tubule is extensively convoluted and an appreciable proportion is branched so that they have three openings into the rete testis. The sperm from each testicle then passes through very small tubes into an epididymis, each epididymal tube leads to longer tubes which are the vas deferens. The two vas deferens ducts converge from the left and right sides of the body to connect with the urethra canal at its upper end very near to where the urinary bladder opens into the urethra. The urethra is a large canal that leads through the penile organ to the outside of the body (Ogbuewu, 2008).

II.2.1. Puberty and Sexual Maturity:

Rabbits are well known for their ability to reproduce quickly. The onset of puberty varies greatly with breed but conditions in rabbit breeding also play an essential role, particularly feeding, which is even more important than climate (Lebas *et al.*, 1997).

Around six (6) weeks of their age, there is a quick increase in Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) concentrations in the blood which precede the onset of testosterone secretion and consequently the manifestation of puberty (Chubb *et al.*, 1978). Skinner (1967) reported that rabbits are pubertal when their testicles become androgenically active and accessory glands begin to produce fructose and citric acid then the animal is said to characteristically assume male behavior. He affirmed that at 63 days of age, rabbit testes descend into the scrotum. Studies revealed that although rabbits are pubertal in 4 months, the testes are not in the scrotum yet, as the descending into the scrotum is observed only at six months of age according to Fraser (1988).

II.2.2. Sexual Maturity

Sexual maturity is defined as the moment at which the daily spermatozoa production ceases to increase (Lebas *et al.*, 1997). A buck attains sexual maturity when its daily sperm production stabilizes and becomes able to fertilize the females (Lebas *et al.*, 1986). This is witnessed at the end of the 32nd week of existence in the New Zealand White breed raised under temperate environment (Lebas *et al.*, 1986). In rabbits, sexual maturity varies with age (125 - 150 days), breed, food/feeding and environmental factors such as photoperiod, temperature and seasonality. Sexual precocity is more developed in small or medium breeds (4 - 6 months) than in larger breeds (5 - 8 months). Studies have shown that the New Zealand White reaches sexual maturity at 18 weeks of age (Chubb *et al.*, 1978; Frame, 1994). This is maintained first as the rabbit attempts riding at 70 - 90 days after birth. It is argued however, that the ejaculates are low in viability hence bucks of about 135 - 140 days of age should be used for mating when the viability of the ejaculate is believed to have stabilized (Egbuka, 1995).

II.2.3. Spermatogenesis

Spermatogenesis begins with the formation of spermatogonia stem and the life-time of such stem cells is a cycle of seminiferous epithelium. Spermatogenesis in rabbits starts at 7 - 8 weeks (between 42 and 63 days of age) by the division of spermatogonia but semen do not appear in ejaculated sperm before 119 days according to Skinner (1967). Spermatogenesis is a process that depends on the low temperature of the scrotum thus temperatures higher than that of the scrotum (e.g. abdominal temperature) may block the process (Hua *et al.*, 2000).

The total estimated duration of spermatogenesis in rabbits depends on the point chosen as the beginning of spermatogenesis. Assuming that spermatogenesis begins with the first part of a series of division of spermatogonia leading to the production of primary spermatocytes, then about four cycles of the seminiferous epithelium ($4 \times 10.9 = 43.6$ days) are required. Swierstra and Foote (1963) reported that during spermatogenesis there is considerable loss of spermatogenic cells in the rabbit and that most of this loss occurs during and immediately after the two maturation divisions. However, recent studies have demonstrated the presence of round spermatids in the epididymis (sloughing of spermatids). In other words, they leave the testes before maturation (Zhang, 2002). The last authors also suggested that the age of the animal and season contribute to the sloughing of spermatids which may occur more frequently after puberty or when spermatogenesis begins to occur in an active form.

II.2.4. Sperm Production

Sperm production begins at puberty. The testicles continue to grow and increase sperm production until six months of age (Morton, 2006). The volume of semen ejaculated is about 0.3 to 0.6 ml. Concentration is evaluated at 150 to 500×10^6 spermatozoa per ml, but both volume and concentration are liable to vary. Maximum spermatozoa production is obtained by using the buck regularly once a day. If the buck is used regularly twice a day, each ejaculate has only one half the concentration of spermatozoa. On the other hand, if bucks service several times a day, one day a week, the three or four ejaculates may be concentrated enough to effect fertilization (Lebas *et al.*, 1997). Spermatozoa can already be present in the cauda epididymis at around 15 weeks of age (Chubb *et al.*, 1978).

Daily spermatozoa production is roughly 150 to 300 million, independent of the rate of ejaculation. It is also noted that daily spermatozoa production increases from 15 to 52 weeks of age (Lebas *et al.*, 1997). However, daily exposure to a continuous 14 hour light period negatively affects gonadal reserves (Orgebin-Crist, 1968).

According to the author, under normal conditions, the average yield is 147.4×10^6 /day and 1 g of testis produces 26.5×10^6 spermatozoa/day. However, if the animal is subjected to a rate of two weekly collections, the daily release of spermatozoa in the ejaculate is consistently lower than the testicular production, indicating that approximately 50% of the spermatozoa produced are reabsorbed (Holtz and Foote, 1972).

Different daily production of spermatozoa has been observed: $148 \pm 11 \times 10^6$ spermatozoa per day [34], 187×10^6 /day [42] and 210×10^6 /day (Amann and Lambiase, 1969). A recent study showed that in the New Zealand White breed, the spermatozoa reserve is smaller in the left testis and left epididymis than in the right ones according to Ewuola and Egbunike (2010).

II.2.5. Sperm Motility

Percentage of spermatozoa moving steadily in a straight line for species with internal fertilization is called motility and it is a common feature of spermatozoa in all animals (Chrenek *et al.*, 2007). Motility is important for the transport of spermatozoa in the reproductive tract and oocyte penetration (Holt *et al.*, 2004). Subjective estimation of the evaluation of motility and sperm morphology are the two most widely used laboratory tests to evaluate semen in rabbits (Lavara *et al.*, 2008). These characteristic results in potential spermatozoa fertility as there are some correlations between seminal parameters with motility indicating the relationship between morphometric parameters and semen quality of rabbits (Hagen *et al.*, 2002).

II.2.6. Semen Characteristics

Rabbit semen consists of two main parts; a fluid and a gelatinous portion (Mukherjee *et al.*, 1951). The semen is a mixture of spermatozoa produced by the testes and seminal plasma secreted at the time of ejaculation by the epididymis and different accessory glands combined (El-Azim and El-kamash., 2011). The fluid portion of the semen is represented as the seminal plasma. It contains constituents such as carbohydrates, lipids, proteins and minerals that are important for sperm metabolism. Their presence positively affects the survival and parameters of spermatozoa motility in rabbits (Castellini *et al.*, 2000; Zaniboni *et al.*, 2004).

Rabbit seminal plasma contains sodium (Na), Potassium (k), Calcium (ca), Magnesium (mg), Selenium (se), Zinc (zn) (Castellini *et al.*, 2004) and some trace elements such as Copper, iron, Manganese, Cadbuim, Lead and Nickel (Lukáč *et al.*, 2009). The gelatinous mass or gel from rabbit semen originates in the vesicular (Del Niño Jesus *et al.*, 1997), and it is androgen-dependent (Bell and Mitchell., 1984). It contains a significant amount of oestrogenic substances, citric acid and small amounts of fructose Holtz and Foote (1978) and Mukherjee (1951). International Rabbit Reproduction Group (2005) recommends that the gel be removed immediately after collecting the rabbit semen before its evaluation.

II.2.7. Semen Volume and Sperm Concentration

Sperm concentration and volume of semen ejaculated in rabbit ranges from 150 - 500 × 10⁶ sperm/ml and 0.3 - 0.6 ml respectively (Lebas *et al.*, 1997) (Adams and Singh, 1981).

Some factors such as diet, collection frequency, age, sequence of ejaculate and ambient temperature (Finzi *et al.*, 1994) affect semen volume and sperm concentration. García-Tomás *et al.* (2008) and Roca *et al.* (2005) reported that semen volume seems to be more affected by temperature than sperm concentration. According to Campos *et al.* (2014), Semen composition and volume are influenced by the size of the accessory gland which is in turn influenced by testicular testosterone production among other factors.

This can be explained by the report of El-Azim and El-kamash (2011) who stated that semen is a mix of spermatozoa produced by the testes and seminal plasma secreted by the epididymis and different accessory glands, of which functions are combined at the time of ejaculation, also according to the report of Hafez (1995), the accessory glands contribute to the greater part of the volume of ejaculate. It therefore means that, if these organs are small in size as a result of poor development during the animal's growth period, the composition and volume of sperm that will be secreted in the epididymis and other accessory glands which will be produced alongside with testicular testosterone by a small testes, will be influenced when compared with the production of sperm from a well-sized developed reproductive organs. However, other studies reported that there is positive correlation between the gonadal reserve and testicular weight (Orgebin-Crist., 1968) and body of the rabbit (Ewuola and Egbunike, 2010).

II.2.8. Semen Colour

There are some ratings for the colour of rabbit semen according to different re-searchers though several studies have associated color and appearance as a single parameter (Scapinello *et al.*, 1997; Mataveli., 2008), and El-Azim and El-kamash (2011). By International Rabbit Reproduction Group (2005), normal semen is white, homogeneous and opalescent. According to Alvarez *et al.* (2006), the colour of rabbit semen is white with the intensity dependent on the concentration of the sperm.

He further reported that milky-white semen is the best and predominant in the rabbit and represents normal semen with good quality, while Mataveli (2008) reported that rabbit ejaculate is mostly milky-white but the best quality is found in creamy-white semen. For Bilbao (1996), the semen is often pearly white and ivory, but gray semen is considered of poor quality. Also from the research study of Arrebola and Fernández (2011), they reported that rabbit semen is pearly-white which denote good quality while other colours are classified as poor likewise as a uniform appearance is most desirable. According to Chang (1959), yellowish semen is often contaminated with urine that is normally obtained when the temperature is too high in artificial vagina.

II.3. Factors affecting semen production

Different factors such as year, season, frequency and type of collection and its rank, lighting programmes, buck, age and health as well as feeding strategies, can all influence sperm production. According to the study of International Rabbit Reproduction Group (IRRG, 2005) that have analyzed these factors, only genetic strain and feeding will be discussed here.

II.3.1. Genetic component

It is known that the genetic strain can influence the semen production and the spermatozoa characteristics (Amann, 1966; Abo El-Ezz *et al.*, 1985; Dubiel *et al.* 1985). However, only recently some papers have been published that gave the results of long term studies of the semen characteristics of bucks of various genotypes (Brun *et al.*, 2002a, Theau-Clément *et al.*, 2003, Moce *et al.*, 2005). For example, Bencheikh (1993) compared the INRA-A1077 strain (New-Zealand origin) and the INRA-A2066 (Californian origin): semen from the former collected once a week (2 ejaculates within an interval of 15 minutes) produced almost twice as much semen of better quality.

The use of crossbred males can improve sperm production: Brun *et al.* (2002a) found evidence of a significant heterosis effect for concentration (+37.5% of the parental average), mass motility (6.8 %) and the percent of motile spermatozoa (4.1 %). However, Garcia *et al.* (2004) observed a high heterosis only for the presence of distal cytoplasmic drops (35 %). Brun *et al.* (2004), studying the effects of divergent selection on body weight (heavy and light lines), assessed certain differences in seminal traits and hence a genetic relationship between body weight and semen production. The number of high quality spermatozoa per ejaculate (number of motile sperms/ejaculate and their ability for insemination), was higher in the light line.

Differences between bucks may arise both from genetic and environmental origin: however, when bucks of a selected strain are reared and collected according to a very strict protocol, the variability is generally lower than that obtained in less controlled conditions (Bencheikh, 1993 Vs. Panella and Castellini, 1990).

II.3.2 Feeding strategies

Several centres for the production of semen have been created within Europe and have permitted the development of farming practices adapted to rabbit bucks. While for some factors detailed data exist, dietary recommendations (de Blas and Wiseman 1998) are not yet available.

However, it is reasonable to assume that improvements in the quantity and quality of semen are mainly affected by the lipid and the antioxidant diet profile, which in turn influences the behaviour, and the characteristics of the spermatozoa membrane.

II.3.2.1 Fat

Providing of a balanced fatty acid composition seems to be very important for obtaining high quality semen. A very large amount of spermatozoa lipids are polyunsaturated (PUFA) of n-3/n-6 series (Apel-Paz *et al.*, 2003) and these fatty acids modify the membrane fluidity and its properties. The fusogenic property of spermatozoa membrane is very important during capacitation, AR and fusion with the oocyte membrane. Increasing dietary PUFA thus increments their respective levels in the membrane and improves the kinetic traits of rabbit spermatozoa (Castellini *et al.*, 2004).

II.3.2.2 Antioxidant

The high unsaturation of spermatozoa lipid membrane renders these cells very susceptible to peroxidation, which affects membrane structure, physiology and DNA integrity. Antioxidant protection of spermatozoa is assured by several compounds in the seminal plasma (enzymes, albumin, urate, tocopherols and ascorbic acid (Mann and Lutwak-Mann, 1981) and is partly affected by their content in the diet. Dietary PUFA supplementation therefore endangers the antioxidant equilibrium of semen, which must be restored by the administration of antioxidants.

One of the more widely used molecules is the α -tocopherol: rabbit bucks fed supra-nutritional levels of antioxidants (200 mg/kg α -tocopheryl acetate and 0.5 g/L vitamin C; Castellini *et al.*, 2000a), show a lower semen lipo-peroxidation. These trends are more pronounced in semen submitted to storage or when diets contain high PUFA levels.

Zaniboni *et al.*, (2004), showed a high level of α - and δ -tocopherol in spermatozoa followed by a progressively lower proportion in droplets and seminal plasma. The possibility that the d-isomer could be a more active antioxidant for rabbit semen is being studied.

Under field conditions, the antioxidant equilibrium is even more important due to the non-perfect hygienic conditions that could produce infection/inflammation of the reproductive apparatus, which has a negative effect on both testicle functions and semen characteristics (O'Bryan *et al.*, 2000) by affecting the biosynthesis of pro-inflammatory eicosanoids, cytokines (Knapp, 1990) and reactive oxygen species (ROS - Jones *et al.*, 1979).

PART TWO:

EXPERIMENTAL

WORK

The knowledge of the study region characteristics is an essential element in any investigation concerning living beings, in particular the case of our study where the geographical location and the environmental factors have a significant impact on the reproduction of rabbit bucks. This chapter is devoted to the presentation of the geographical situation, the climate and the livestock management in the wilaya of Tiaret.

1 Study region

1.1 Geographic location

Tiaret region is located in the west of the country, it is a contact area, between the north and the south, forming part of the high plains. The territory of the wilaya is made up of mountainous areas in the north, high plains in the center and semi-arid areas in the south. This heterogeneous character of the space denotes the variety of the agricultural landscape and the diversity of its landforms (Guemour, 2011).

It extends over a space delimited between $0^{\circ} .34'$ to $2^{\circ} .5'$ East longitude and $34^{\circ} .5'$ to $35^{\circ} .30'$ North latitude. Elongated with a north-south orientation, it covers part of the Tell Atlas to the north, and the highlands to the center and south. The altitudes vary from 500 m (near Oued Lili 850) to over 1200 m (Djebel Guezoul massif) (Guemour, 2011). The territory of the wilaya, occupies a total area of 20,050.05 km² mainly agricultural, with 969,375 ha of total agricultural area (Guemour, 2011).

1.2 General climatic characteristics

The region of Tiaret by its geographical position, the diversity of the forms of its relief, undergoes climatic influences combined with the large masses of air, the exposure of the relief, and the altitude. Indeed, during the winter season, the cold air masses coming from the Atlantic meet the hot and humid air masses, which causes instability and climatic disturbances at the origin of the sometimes intense winter rains (Guemour, 2011)

Throughout the cold and humid season between the months of November to February, the influence of polar air masses contributes to the drop in winter temperatures. During the summer season the tropical air masses linked to the bark anticyclone predominate and cause a high pressure zone causing a type of dry and sunny climate that lasts until the end of September and sometimes even at the beginning of October (Guemour, 2011).

Between the two seasons (during spring and autumn), Saharan air influences are frequently manifested by dry and hot winds (sirocco), causing a significant increase in the evaporating power of the atmosphere and thus causing heat stroke harmful to plants (scalding phenomenon) (Guemour, 2011).

These general climatic fluctuations follow the classic pattern of a Mediterranean-type climate, characterized by two great seasons; one hot and dry which often lasts from May to September and the other cold and more or less humid; which lasts from October to April (Guemour, 2011)

2 Livestock management

The study was conducted at the experimental farm of Ibn khaldoun University of Tiaret, south of the town of Tiaret about 10 km from the Faculty of Nature and Life Sciences (35°20'02.3"N 1°18'41.8"E) western of Algeria from January 2019 to march 2021 .

2.1 Housing

The hutch is located in a favorable location for breeding. The hangar, with a metallic frame has an area of approximately 240 m², faces west. Animals were housed individually in wire cages (20 cages) arranged in flat-deck layout on one level. Automatic waterers were used (drinking nipples). The hutch also has a food storage room. Ventilation and lighting were natural (provided by windows).

The building was thoroughly cleaned and disinfected with bleach and lime before the start of the experiment. The building was subsequently cleaned manually on a weekly basis (elimination of droppings, cleaning with detergents) and monthly for the cages using fire (blowtorch). A footbath was installed at the entrance to the hutch to prevent contamination from outside.

2.2 Animals

Rabbit bucks used in the experiment were the product of a crossing up to the 5th generation between local population rabbits gathered from different farms in the region of Tiaret.

The rabbits were chosen according to their availability. The crossing were made in the experimental farm.

During the whole experimental period, both feed and water were administrated ad libitum. Feeding was with a commercial diet of 13,81% of crud protein and 2820 kcal digestible energy/kg. (Table of ingredients in annexe).

2.3 Medical prophylaxis

In our experiment we have used a prevention against diseases by the active ingredients and vitamin complexes associated with trace elements. Thus, the preventive treatment against coccidiosis, most often carried out using oral anticoccidial drugs (Agicox) and a vaccine against enterotoxemia in a subcutaneous injection at the 8th week of age (by using 1 ml of Coglavax)

In addition to a preventive treatment against scabies and external parasites (with Ivermectin), vitamin complexes used regularly have been incorporated into the water.

CHAPTER I:

SPERM QUALITY IN RESPONSE TO AGE IN LOCAL ALGERIAN RABBIT BUCKS

1. Introduction

The best rabbit's performances use depends entirely on good reproduction management. According to Alvariño (2000); male is the basis of breeding success playing a key role in the achievement and profitability of the rabbit's breeding. The buck in natural breeding is used to fertilize 8 to 11 does (Roca, 1994; Osechas & Becerra, 2006) in contrast by artificial insemination it can be used to fertilize 100 does at a time, because of the fertilizing capacity of semen (Eid, 2008).

Semen evaluation traits informs about the spermatozoa fertilizing capability (Boiti *et al.*, 2005). Moreover, there are a wide variety in semen traits, and different factors such as the collection frequency, lighting programs, buck age, might influence qualitative and quantitative sperm production (Boiti *et al.*, 2005). To obtain an optimal quantity of sperm and spermatozoa, it is necessary to define the conditions of use of the bucks (Boulbina *et al.*, 2012). The analyses of sperm production is significantly highly correlated with sexual activity (Benia *et al.*, 2018), it is necessary to identify the puberty age, sexual maturity, the response to the collect and factors affecting the sperm production.

In order to characterize male reproductive performance of the Algerian local rabbit population; the aim of this study was to assess the sperm quality evolution with age in local rabbits raised in the semi-arid environment.

2. Materials and methods

2.1. Location

The study was conducted at the experimental farm of Ibn Khaldoun University of Tiaret (western Algeria) during 2019. The farm is situated in the west of the state characterized by a cold weather in winter and very hot in summer.

2.2. Animals

Rabbit bucks were the product of a crossing between local populations (rabbits gathered from different farms from the Tiaret region) up to the 5th generation. Twenty rabbit bucks of the local population weighting between 3010 – 4540 were collected (from the 5th to the 11th month of age) to evaluate the semen quantity and quality evolution.

2.3.Housing

Animals were housed individually in wire cages arranged in flat-deck layout on one level. Ventilation and lighting were naturally provided. Automatic waterers were used, and the rabbits were fed ad libitum with granulated commercial diet (13,81% crud protein, 2820 kcal digestible energy/kg).

2.4.Semen collection and analyses

2.4.1. Preparation of artificial vagina

The AV is prepared by filling the water jacket with water hot enough to result in a final AV temperature of 42° to 50° C. Air can be added to increase the pressure until the AV liner bulges out of the end slightly. Finally, the AV is lubricated with a sterile nonspermicidal gel (Barth, 2007).

2.4.2. Semen collection

The semen collection was made by an artificial vagina, introducing a teaser doe to the male cage. When the buck mounts, the collector must be ready to step in immediately and direct the penis to the opening of the artificial vagina and collect sperm. In this experiment we have used an extensive rhythm of collection. After collection we have analyzed the sperm. Some samples were used in artificial insemination.

2.4.3. Studied parameters

Libido was recorded in terms of reaction time in seconds and was estimated from the time the doe was placed inside the buck's cage up to the point when the buck started to mount the doe (Daader *et al.*, 1999). After collection, the volume of the ejaculate was assessed by reading the graduation of the collecting tube. Sperm volume is deducted after removal of the gel fraction. The collection tube was immediately put into an electric oven at 37 °C. The pH of the semen is determined by a pH paper. Then, the mass (MM) and individual (IM) motility of the spermatozoa were determined under a phase contrast microscope. Mass motility was appreciated by placing a drop of pure sperm between slide and lamella observed at magnification (x10), a note from 0 to 9 was attributed to the movement of sperm mass observed on the Petitjean scale (1965) mentioned by (Boussit, 1989). Individual motility was assessed after dilution of the sperm with a commercial diluent at the rate of 1/5 and 4/5 diluter volumes. A drop of diluted semen was observed

between slide and lamella at magnification (x40), a note from 0 to 4 was attributed to the individual movement of spermatozoa observed on the Adrieu scale (1974) according to (Boussit, 1989).

The concentration (C) in spermatozoa ($10^6/\text{ml}$) was determined using a malassez cell from a drop of seed diluted to 1/200 with the diluter. Counting was performed under the microscope at magnification (x40) (Boussit, 1989).

The vitality was determined by the preparation of a smear using eosin-nigrosine vital staining, a drop of semen was mixed with a drop of the dye, and then the mixture was gently spread along the blade. The smear was left for a few seconds, then it was observed under magnification microscope (x100). Dead sperms spread the dye through their damaged membrane, while living spermatozoa with their functional membranes do not diffuse the dye and therefore remain colorless. A random count of 150 spermatozoa was performed along the smear, from which dead spermatozoa were distinguished from the living according to the Adrieu scale (Boussit, 1989).

The percentage of abnormal spermatozoa (AS %) was studied on the same sample of the stained smear. 150 spermatozoa were randomly counted and abnormal spermatozoa were distinguished (Boussit, 1989).

2.5. Statistical analysis

Data were collected and statistically analyzed using one-way ANOVA (IBM® SPSS 25 software). The variables analyzed were macroscopic sperm parameters (weight, Libido, volume, and pH) and microscopic parameters (mass motility, individual motility, concentration, vitality, and percentage of abnormal sperm) and the effect of age on these parameters.

3. Results and discussion

In this work, a total of 103 semen samples from the 5th month until the 11th age were collected and analyzed Table 1.

Age (Months)	5 (n=10)	6(n=15)	7(n=16)	8(n=17)	9(n=16)	10(n=15)	11(n=14)	Total (n=103)
weight (g)	3557±367,91	3675±332,70	3745±336,94	3858±354,53	3955±304,10	3814±287,52	3736±354,32	3777±331,16
Libido (s)	33,7±16,29	26±19,01	23,38±27,29	34,82±26,97	24,56±19,83	16,27±12,80	19,14±12,98	25,17±20,94
pH	7,13±0,26	7,10±0,20	7,52±0,30	7,61±0,50	7,38±0,30	7,59±0,34	7,67±0,36**	7,44±0,39
Volume (ml)	0,98±0,45	1,48±0,33*	1,44±0,33	1,12±0,59	1,03±0,36	1,05±0,28	0,99±0,27	1,17±0,43
Concentration (10%/ml)	314±52,54	412±86,45	405±94,52	426±158,39	456±175,15	548±151,15	599±148,94*	456±154,43
Mass motility	6,5±0,71	6,27±0,80	6,38±1,36	6,59±2,45	7,19±2,04	8,07±1,10	6,86±1,79	6,84±1,70
Individual motility	2,8±1,03	3,13±0,64	2,88±1,20	2,41±1,18	3,19±1,17	3,4±0,74	2,93±1	2,96±1,04
Abnormal sperms (%)	46,5±6,54	41,13±6,66	38,25±8,06	29,29±12,67	30,69±7,58	39,33±8,06	47±3,40*	38,17±10,21
Vitality (%)	53,05±18,11	46,53±8,90	58,94±10,36	60,71±23,53	66,5±21,08	75,87±9,69	64±14,66	61,18±18

*Refers to a significant difference ($p<0,05$). **Refers to a very significant difference ($p<0,01$).

Table 1. Mean±SD values of the semen parameters analyzed

3.1.Libido and pH of semen

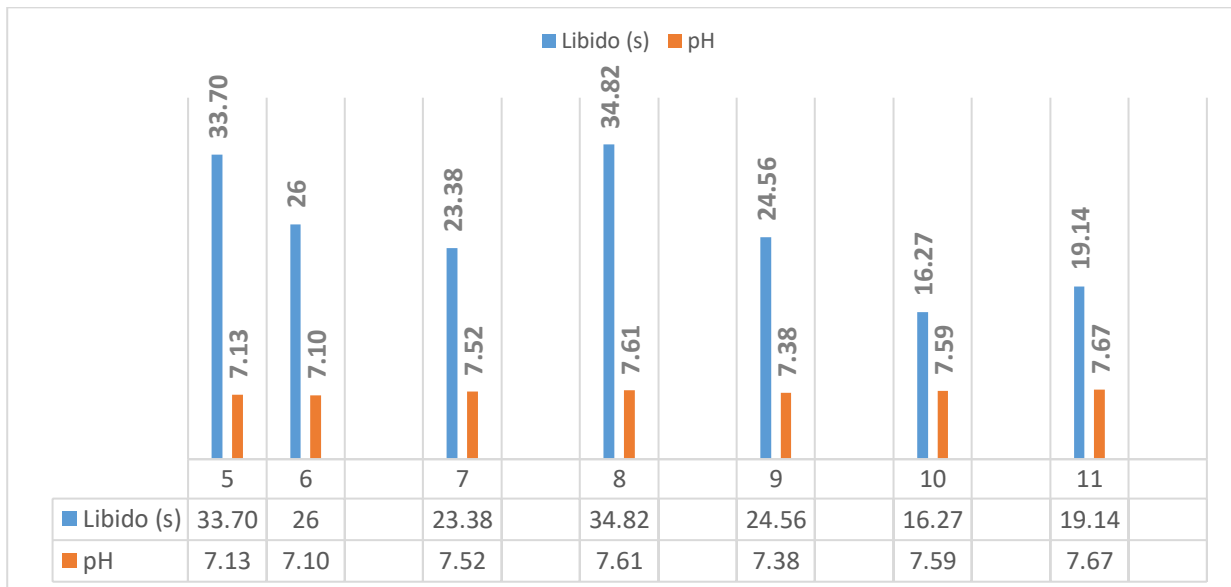


Figure 4. Libido and pH variation with age of bucks

In our work, the average value of libido was $25,17\pm 20,93$, lower than 14,5 and 21,9 respectively reported for the Black Baladi and White New Zealandies rabbits by Safaa *et al.* (2008). These differences appear to be related to the genetic origin of the rabbits and the breeding program to which they have been subjected (Lankri *et al.*, 2019). While the pH of semen were significantly higher ($p<0,05$) at 11th month of age with $7,67\pm 0,36$, this can be due to the increased secretions of the vesicular glands (Rigal, 2008).

3.2. Volume of ejaculate

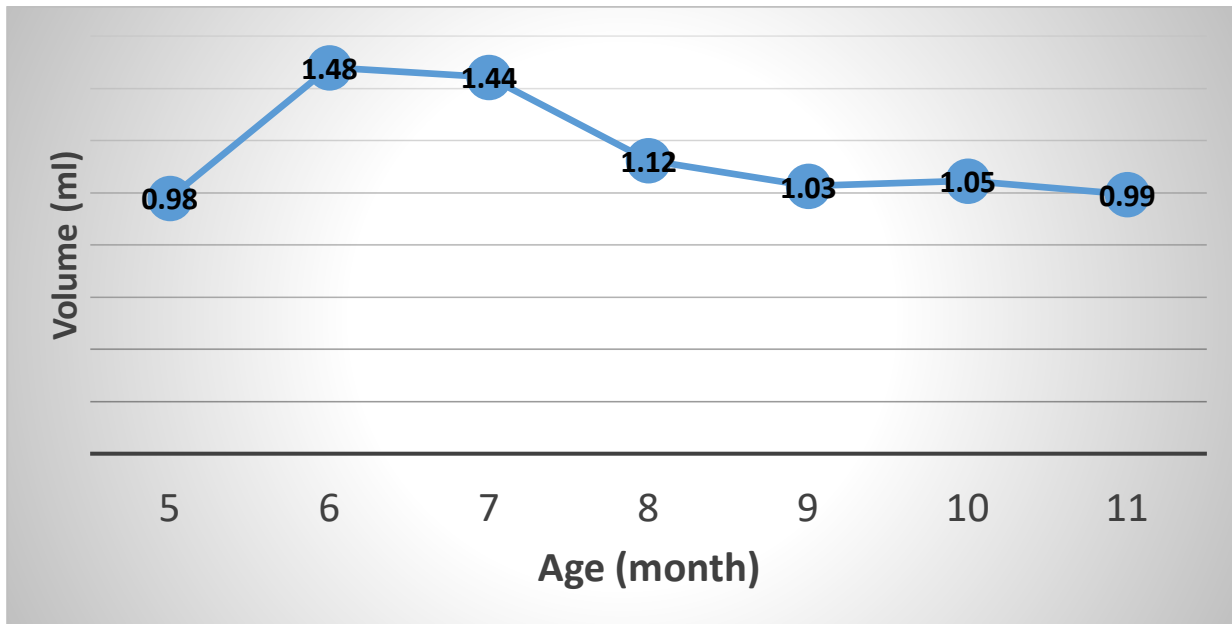


Figure 5. Effect of bucks age on ejaculate volume

The ejaculate volume of the rabbits' semen, in this work was about $1,17 \pm 0,43$ ml, higher than values from 0,3 to 1 ml reported by Orgebin-Crist (1968), Cole and Copps (1977) and Lebas *et al.* (1996) in adult rabbit sperm, and Boulbina *et al.* (2011) with 0,86 ml on the local rabbit population. Feeding Ad libitum increase the libido and the volume of the sperm according to (Alvarino, 2000). In the other hand; the volume of the semen, gel fraction, sperm motility, sperm concentration and morphological alterations, show high variations among the different breeds (Dubiel *et al.*, 1985; Abo El-Ezz *et al.*, 1985). The highest semen volume was recorded at six months of age which coincided with March and according to Boussit (1989) the volume of ejaculate reaches its high values from March to June.

3.3. Concentration of spermatazoa

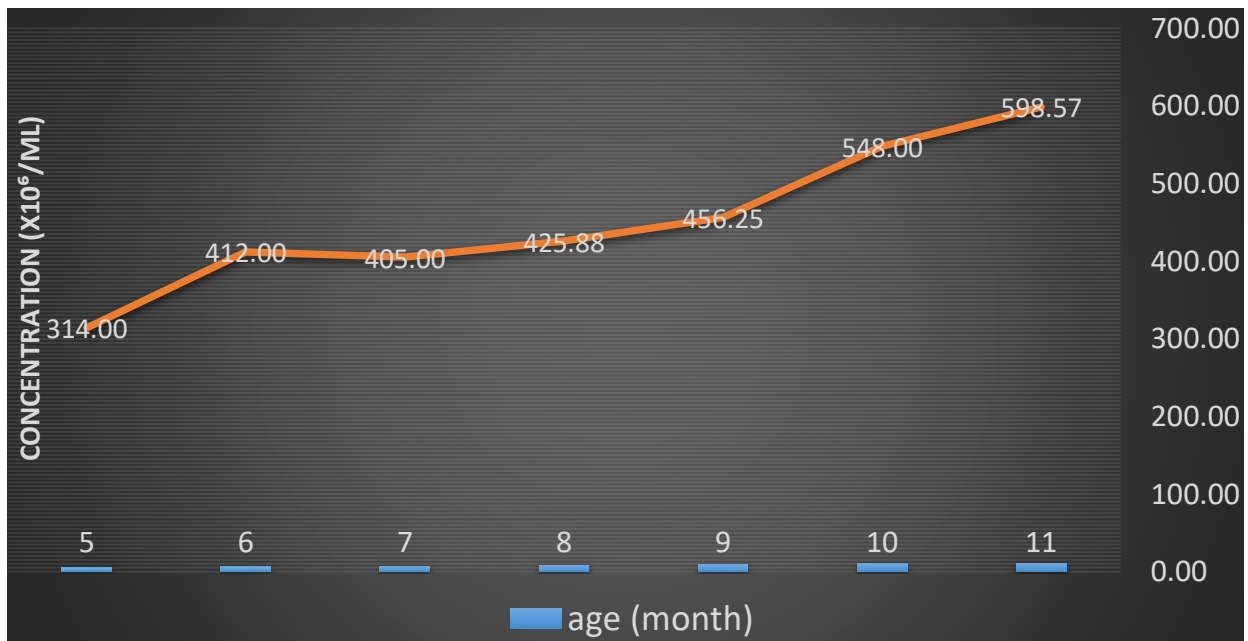


Figure 6. Concentration variation with age of bucks

Joly and Theau-Clément (2000) reported that the biological characteristics of the semen (volume, concentration, motility, morphological alterations ...) are very variable between and intra-breeds, but on average the values of these parameters increase with the age of bucks (from 5 months to 24 months). In this work, the adult's semen concentration was significantly higher with $599 \pm 148,94 \times 10^6/\text{ml}$ in the 11th month of age. According to Theau-Clement *et al.* (2003) the age of males influences significantly the sperm concentration and production. Our results are higher than $232 \times 10^6/\text{ml}$ and $220 \times 10^6/\text{ml}$ reported by Safaa *et al.* (2008b) of two selected lines of New Zealand rabbit bucks, otherwise, it was recommended to make a collect one a week (Tacke *et al.*, 1995; Bencheikh 1993; 1995), however, our rabbits were collected 2 to 3 times in a month. The different studies were based on observation and counting, which are probably an additional source of variability in the results (Cabannes, 2008).

3.4. Sperm motility

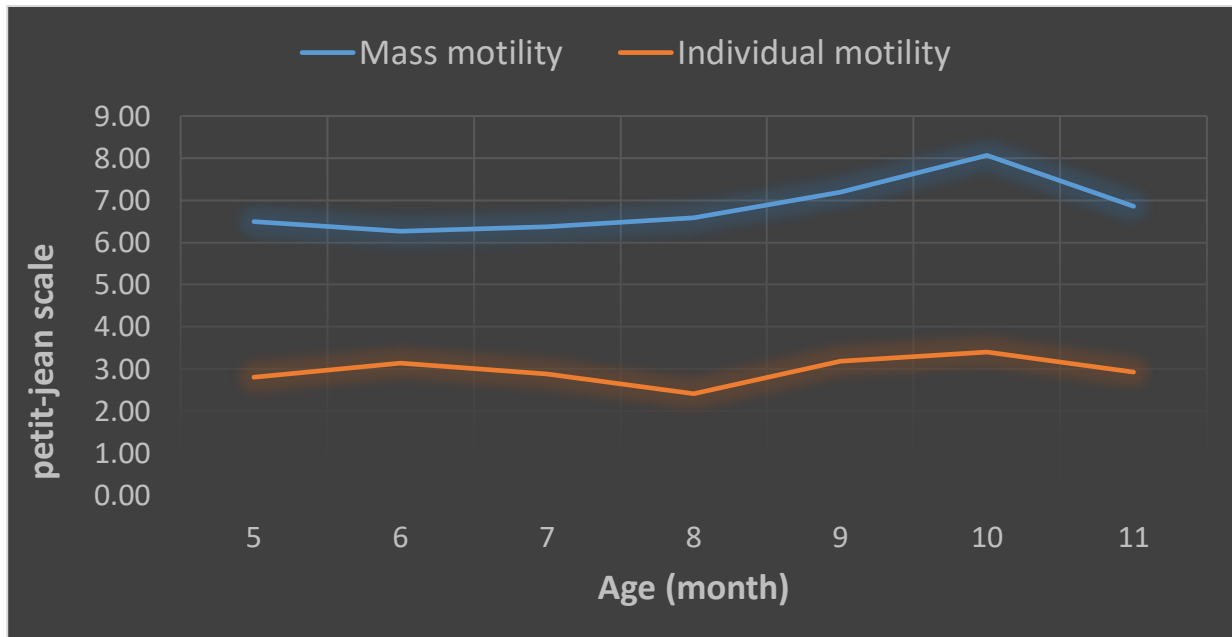


Figure 7. Mass and individual motility according to bucks age

Sperm motility is a very important parameter that reflects the quality and has a significant effect on egg cell fertilization (Wysokińska *et al.*, 2013). The higher rate of motility in this work was recorded in the 10th month of age. However, the average of total values of mass and individual motility were about 6,84 and 2,96 respectively, lower than values reported by Boulbina et al (2011) in male rabbits of the local population with a mass motility of 7,68 and an individual motility of 3,57. It was estimated that semen had a good mobility with mass motility with an appearance of waves ($\geq 6/9$) and individual motility with a fast progression ($\geq 3/4$) (Boussit, 1989).

3.5.Vitality and abnormal spermatozoa

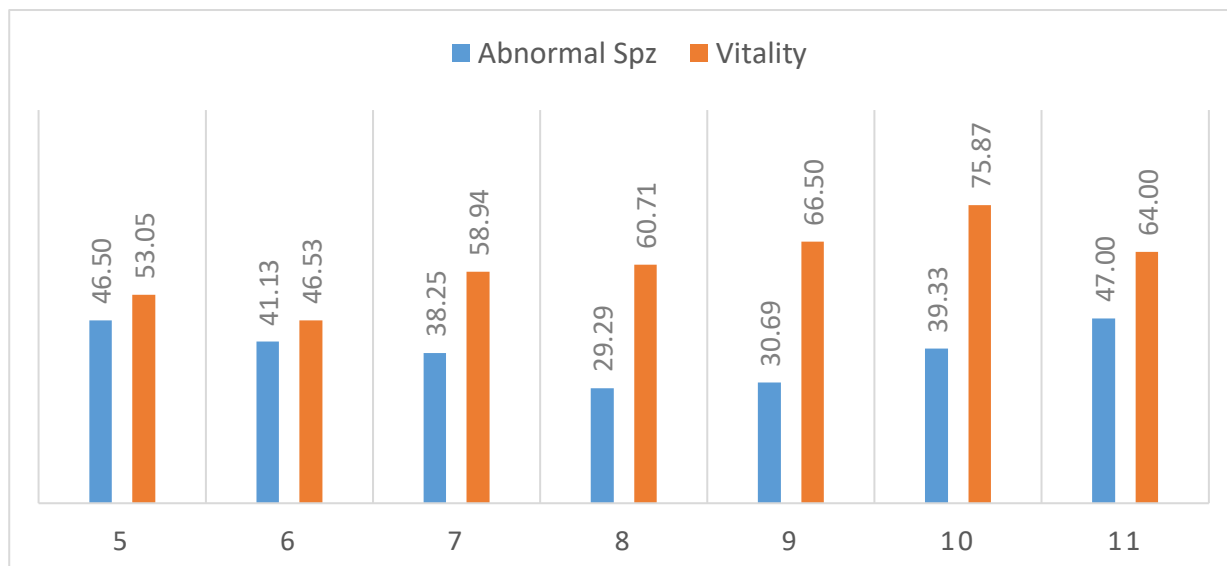


Figure 8. Age effect on vitality and abnormal spz rate (%)

The vitality rate of the sperm collected increased with age and the highest value was recorded at 10th months of age with $75,87 \pm 9,69\%$. According to Boulbina et al (2012) delayed puberty gives more time for testicular and epididymal function to settle, and consequently a higher quality semen from the first ejaculates. Moreover it start decreasing after the 10th month because semen quality generally decreases in older rabbit bucks (Castellini, 2008).

The total sperm abnormalities rate was significantly higher at 11th months with a value of $47 \pm 3,40\%$, it was explained by the high environmental temperatures recorded at the moment of collection, because the age of 11 months coincided with August. However, a significant interaction was observed between the breed and season (Safaa *et al.*, 2008a).

4. Conclusion

It could be concluded from the current study that the age has an important effect on semen production and its different characteristics.

Semen analyses in this study and its use in artificial insemination has shown that the Algerian local bucks remain good and desirable for reproduction both natural breeding and artificial insemination.

It appears that rabbit local population bucks of 10th month of age are the best and desirable breeders.

We recommend to improve livestock under controlled conditions to have better results.

CHAPTER II:

SPERM CHARACTERISTICS VARIATION OF ALGERIAN RABBIT BUCKS UNDER DIFFERENT TEMPERATURES

1. Introduction

Rabbit meat production remains low in Algeria, even though it's a precious source of meat and protein of lower cost. The improvement of this production needs a better reproduction administration. Male plays a key role in the success and profitability of breeding (Alvarino, 2000), since numerous does are bred by a single buck. The male fertility evaluation prior to breeding is paramount of importance to achieve breeding success (Ranjan *et al.*, 2020).

The selection of adequate bucks depends on sperm assessment which acquires the efficiency of reproductive performance. Thus, semen evaluation must provide information on spermatozoa fertilizing capability (Boiti *et al.*, 2005).

To obtain an optimal quantity of sperm and spermatozoa, the conditions of use of the bucks have to be defined, so, it is necessary to identify its response to the collect and the factors of variation influencing the sperm production (Boulbina, *et al.*, 2012). Moreover, the heat stress (HS) is the main factor that elicits notable alterations in the testes, which ultimately alters sperm structural and functional integrity (Maya-Soriano *et al.*, 2015; Pei *et al.*, 2012). Also, temperature essentially influences semen motility (Hahn *et al.*, 2019).

The aim of this work was to evaluate the sperm production characteristics evolution within temperature in Algerian local rabbit's population raised in the western region under semi-arid environment.

2. Material and methods

A total of 102 samples were analyzed in different temperatures between 10°C and 32°C collected from twenty adult rabbit bucks of the local population weighting between 3010 – 4540 g.

Rabbits were housed individually in wire cages arranged in flat-deck layout on one level and fed ad libitum with granulated commercial diet. Automatic waterers were used. Ventilation and lighting were natural.

Daily ambient temperature was recorded in degree celcius (°C) using a thermometer.

An extensive rhythm collection was used for 6 months to evaluate the semen quantity and quality evolution within different temperatures. The collect was made using an artificial vagina, using a teaser doe at collection time. Ejaculates with urine or blood drops and ruined samples were discarded. Libido was timed in seconds; from the time the doe was placed inside the buck's cage up to the point when the buck started to mount the doe.

Immediately after collection, the volume of the ejaculate was assessed by reading the graduation of the collecting tube. Sperm volume was deducted after removal of the gel fraction. The collection tube was immediately transferred into an electric oven at 37°C.

In this work, the effect of temperature on libido, sperm volume and pH, mass and individual motility, concentration, vitality and abnormal sperm rate in local rabbits were determined.

pH was determined using a pH paper. Mass motility (MM) was appreciated by placing a drop of pure sperm was observed at (x10) magnification, a note from 0 to 9 was attributed in Petitjean scale mentioned by Boussit (1989). Individual motility (IM) was assessed after dilution of the sperm with a commercial diluent at 1/5 and 4/5 diluter volumes. A drop of diluted semen was observed at (x40) magnification, and a note from 0 to 4 was attributed in a scale of Adrieu mentioned by Boussit (1989).

The concentration in spermatozoa (C) was determined using a malassez cell counter from a drop of sperm diluted to 1/200 with the diluter (Boussit, 1989).

The vitality was determined by the preparation of a smear using eosin-nigrosine vital staining, a drop of semen was mixed with a drop of a dye and was gently spread along the blade. The smear was left for a few seconds, then it was observed at (x100) magnification. Dead sperms spread the dye through their damaged membrane, while living spermatozoa remain colorless. A random count of 150 spermatozoa was performed along the smear, from which dead spermatozoa were distinguished from the living ones. The abnormal spermatozoa (AS) rate was assessed on the same sample of the stained smear. 150 spermatozoa were randomly counted and abnormal spermatozoa were distinguished.

Data collected, were studied statistically using the IBM® SPSS 25 software and one-way ANOVA was performed.

3. Results

Ejaculates volume, libido and pH mean values are expressed in Table 02. In this this work, mean term reaction was $24,99 \pm 20,96$ s, the different temperatures had no significant effect on libido while the volume of the ejaculates with $1,17 \pm 0,43$ ml.

In this work, pH was significantly affected by temperature ($p < 0,05$). In 35% of the samples, pH was about 7,7–8,4 in the buck semen, however, 65% of the samples had a decreased pH value of 7,2.

Concentration, mass and individual motility at different temperatures are expressed in Table 03. The mean concentration value was $459,02 \pm 149,62 \times 10^6/\text{ml}$ while the average mean mass and individual motility were respectively $6,91 \pm 1,56$ and $2,99 \pm 1$.

Mass and individual motility in collected samples from males under 25°C were lower ($p < 0,05$) than other temperatures. Concentration was significantly higher ($p < 0,05$) in bucks kept under 27°C and decreased in low temperatures. Living spermatozoa rate was significantly higher ($p < 0,05$) in rabbits kept under 29°C . However, in this work, abnormal spermatozoa increased within temperatures ($p < 0,05$).

Temperature ($^\circ\text{C}$)	Libido (s)	Volume (ml)	pH
10	19 \pm 10,49	1,5 \pm 0,37	7,46 \pm 0,42
12	22,8 \pm 14,96	1,22 \pm 0,26	7,28 \pm 0,19
14	15,29 \pm 8,73	1,21 \pm 0,48	7,2 \pm 0,22
15	26,75 \pm 17,87	1,23 \pm 0,61	6,93 \pm 0,05
16	23,4 \pm 16,64	1,34 \pm 0,59	7,28 \pm 0,13
17	26,67 \pm 17,67	1,23 \pm 0,25	7,03 \pm 0,32
18	37 \pm 31,11	1,45 \pm 0,64	7,25 \pm 0,21
19	36,67 \pm 24,5	1,37 \pm 0,47	7,7 \pm 0,26
20	35,4 \pm 26,52	1,62 \pm 0,26	7,2 \pm 0,45
23	43,71 \pm 36,34	1,07 \pm 0,31	7,51 \pm 0,32
25	27,17 \pm 15,11	1,32 \pm 0,81	7,68 \pm 0,66
26	36 \pm 40,01	1,28 \pm 0,54	7,63 \pm 0,43
27	25,2 \pm 2,6	0,9 \pm 0,28	7,74 \pm 0,42
28	32,1 \pm 29,46	0,89 \pm 0,27	7,44 \pm 0,38
29	12,44 \pm 6,27	1,01 \pm 0,29	7,63 \pm 0,36
30	19,17 \pm 11,72	1,1 \pm 0,41	7,43 \pm 0,29

31	13,27±5,08	1,07±0,34	7,75±0,2*
32	27±19,39	1,08±0,27	7,26±0,33
Total	24,99±20,96	1,17±0,43	7,45±0,39

*indicates a significant difference $p < 0,05$ in the same column.

Table 02. Mean±SD libido, volume and pH values of fresh ejaculate under different temperatures.

Temperature (°c)	Concentration (10⁶ /ml)	Mass motility	Individual motility
10 (n=5)	344±94,23	5,6±1,14	2,6±0,55
12 (n=5)	368±86,72	6,2±0,45	2,8±1,10
14 (n=7)	377,14±94,82	6,71±0,67	2,71±1,25
15 (n=4)	385±114,75	6,5±0,58	3,25±0,5
16 (n=5)	420±96,95	6,8±0,45	3,8±0,45
17 (n=3)	366,67±11,55	7,33±0,58	3,67±0,58
18 (n=2)	280,00	6,00	2,5±0,71
19 (n=3)	446,67±83,27	7,00	3,67±0,58
20 (n=5)	364±118,66	6,2±0,45	2,6±0,55
23 (n=7)	491,43±106,99	7,43±0,98	2,71±0,76
25 (n=6)	420±125,86	5,17±2,93*	1,67±1,37*
26 (n=4)	460±118,88	7,5±1	2,75±0,96
27 (n=5)	620±171,46*	6,4±2,7	2,6±1,34
28 (n=10)	510±102,09	7,4±1,26	3,4±0,84
29 (n=9)	575,56±149,26	8±0,71	3,67±0,5
30 (n=6)	373,33±147,87	7,17±1,47	3±0,89
31 (n=11)	605,45±97,61	8,18±0,87	3,54±0,52
32 (n=5)	416±276,91	5,8±3,11	2±1,41
Total	459,02±149,62	6,91±1,56	2,99±1

*indicates a significant difference $p < 0,05$ in the same column.

Table 03. Concentration, mass motility and individual motility of bucks' semen kept under different temperatures (Mean±SD)

Live sperm percentage value was higher ($p \leq 0,01$) in rabbits kept under 29°C. However, the percentage of abnormal spermatozoa has increased with the live sperm percentage and it was affected by the variation of temperatures ($p < 0,05$).

The mean percentage of viability and abnormal spermatozoa at varying temperatures are shown in Table 04.

Temperature (°c)	Viability (%)	Abnormal spermatozoa (%)
10 (n=5)	51,7±20,32	44,2±8,38
12 (n=5)	54±11,22	45,4±6,23
14 (n=7)	61,71±15,13	36,86±10,87
15 (n=4)	52±1,63	41,75±7,68
16 (n=5)	46±7,62	45,2±7,69
17 (n=3)	46±21,07	46±5,57
18 (n=2)	42,5±27,58	41,5±3,54
19 (n=3)	58±4	40±5,29
20 (n=5)	54±5,48	37,6±4,77
23 (n=7)	70,57±9,43	28±6,63
25 (n=6)	50,33±22,99	36±14,03
26 (n=4)	72,5±13,6	30±9,09
27 (n=5)	61,6±20,85	47,6±3,58*
28 (n=10)	72±10,79	33,8±10,04
29 (n=9)	72,89±13,64*	39,56±8,82
30 (n=6)	63,33±15,93	30,67±6,15
31 (n=11)	72,36±11,79	40,45±8,12
32 (n=5)	58±29,63	42±9,59
Total	61,78±17,03	38,54±9,53

*indicates a significant difference $p < 0,05$ in the same column.

Table 04. Percentage of viability and abnormal spermatozoa evolution within temperatures (Mean±SD)

4. Discussion

It is well known that there is a wide variety in semen traits and different factors that can influence qualitative and quantitative sperm production such as collection frequency, temperature, lighting programs and buck age (Boiti *et al.*, 2005).

In this work, reaction time decreased slightly during high temperatures exposure but no significant difference was observed. Indeed, libido is controlled by many factors, such as hormone, sexual pheromone and the hypothalamus–pituitary–testis axis (Yang *et al.*, 2005). According to Pei *et al.* (2012) the decrease of testosterone in high temperature is consistent with reduced libido under heat stress, which indicate the involvement of testosterone in the regulation of libido.

In our study, no significant differences in semen volumes were recorded between bucks collected under different temperatures. However, with an average volume of 0,86 ml on the local rabbit population was higher than values reported by Lebas *et al.* (1996) and Boulbina *et al.* (2011). According to Alvariño (2000) feeding Ad libitum increase the libido and the volume of the sperm. Extreme heat stress get animal physically exhausted and reduce eagerness which might result in higher reaction time and increase total time for successful ejaculation, thus having an ultimate effect on sperms production (Mandal *et al.*, 2000).

Changes in the semen pH and sperm morphological alterations increases during the summer (Amin *et al.*, 1987) which matches with our findings. The highest values of semen pH were recorded under 31°C, this can be due to the increased secretions of the vesicular glands (Rigal, 2008). Moreover, high temperatures (more than 27 °C) can affect fertility due to increasing semen pH values and morphological alterations, as well as a decrease in sperm motility and libido (Brockhausen *et al.*, 1979; Bagliacci *et al.*, 1987). In this study, we observed a slight decrease in pH of 65% of bucks. This may be attributed to the spermatozoa metabolic activity releasing lactic acid that decreases pH while fructose is the semen major source of energy (Bencheikh, 1995; Klein *et al.*, 1963).

Sperm motility is a very important parameter that reflects its quality and has a significant effect on egg cell fertilization (Wysokińska *et al.*, 2013). In the current study, we have observed that bucks kept under 25°C showed a lower sperm motility. Similarly, in mouse (Pérez-Crespo *et al.*; 2018) with more intense testicular heat exposure (42°C for 30 min), at 14, 21 and 28 days after exposure, there were reductions in both total motility (from ~67% to 28, 8 and 37% respectively) and progressive motility (from ~39% to 5.5, 6.5 and 6.6%). Also, we have noticed that motility

on average was higher in bucks kept under high temperatures comparably with low temperatures ($\leq 15^{\circ}\text{C}$). Furthermore, Llamas-Luceño *et al.* (2020) indicated that total motility of fresh sperm from young bulls was higher ($p < 0,001$) when spermatogenesis occurred in summer under high temperatures compared with other seasons, which agree with our findings.

While, a significant influence of temperature was observed on the proportion of fast-motile sperm, with the better results when animals were kept at 20°C according to Hahn K. *et al.* (2019). The different studies based on observation and counting, which are probably an additional source of variability in the results (Cabannes, 2008).

The Algerian local bucks remain better and desirable for reproduction (natural breeding and artificial insemination) in comparison with other strains (Karim *et al.*, 2020). In our work, values for sperm-cell concentration with $459,02 \pm 149,62 \times 10^6/\text{ml}$ were higher than those reported by Safaa *et al.* (2008b) of two selected lines of New Zealand rabbit bucks with $232 \times 10^6/\text{ml}$ and $220 \times 10^6/\text{ml}$. These differences can be explained by genetic, environmental factors, the different criteria employed for the evaluation and the use of various semen processing technologies according to Safaa *et al.* (2008).

The values of sperm concentration increased with temperatures. Animal in summer season showed highest thermal regulation, which maintain live body weight, improve sexual desire and semen quality under heat stress condition (Abdel-Khalek *et al.*, 2019).

Finzi *et al.* (1995) reported that the daily exposure of rabbits in a climatic chamber to high ambient temperature (30°C) and humidity (70%) for 21 h over a 60 days period increased the number of abnormal spermatozoa. In the current study, the high rate of abnormal spermatozoa was recorded in a temperature equal to 25°C . Although, we have noticed that high temperatures had a less impact in comparison with bucks kept under 25°C . This may be due to the few days of exposure in high temperatures. Generally, when testicular temperature rises, sperm morphology often remains normal for a few days, if sperm in the epididymis are minimally affected, followed by appearance of morphologically abnormal sperm (Barth and Oko, 1989). According to Shahat *et al.*, (2020) heat stress alters sperm quality, with effects on extent and duration of testicular heating.

In contrast, Safaa *et al.* (2008) analyzed the seasonal effects on sperm parameters of Black Baladi and New Zealand's bucks reared in Egypt, and reported that quality parameters such as viability and acrosome abnormalities of semen collected in winter were better than those collected in

summer. These differences appear to be related to the genetic origin of the rabbits and the breeding program (Lankri *et al.*, 2019).

5. Conclusion

Most of the analyzed parameters, that indicates semen quality and predicts its fertility potential, were affected by the variation of temperatures in our study. During high temperatures, which occurs in Algeria in summer, reaction time decreased slightly contrariwise semen pH and sperm morphological alterations increased. According to our finding, better semen can be obtained under moderate temperatures.

CHAPTER III:

MORPHOMETRIC STUDY OF SOME CHARACTERISTICS IN LOCAL ALGERIAN RABBIT BUCKS

1. Introduction

The management of a breeding for production goal must be based in the first place on the knowledge of the species by its identification, from a morphological point of view, and the knowledge of its biological and zootechnical performances. According to Nezar (2007); the morphometric study of the local rabbit would therefore be very important because it would allow not only to bring elements defining in an updated and concrete way this population but also to better analyze its aptitudes.

Mojekwu and Anumudu (2015) declared that morphometrics adds a quantitative element to descriptions, allowing more rigorous comparisons. It enables one to describe complex shapes in a rigorous fashion and permits numerical comparison between different forms. Advancements in morphometrics uses powerful tools for testing and displaying differences in shape, isolate shape from size variation and identifying stocks of specie with unique morphological characteristics.

Morphometrics may be defined as a more or less interwoven set of largely statistical procedures for analyzing variability in size and shape of organs and organisms. Morphometric differences among stocks of a species are recognized as important for evaluating the population structure and as a basis for identifying stocks (Turan; 2004a, Cadrin and Friedland; 1999).

In order to characterize morphometric parameters of the local population rabbit bucks; the aim of this study was to assess the sperm quality evolution with age in local rabbits raised in the Tiaret region Algeria.

2. Materials and methods

2.1. Animals

Sixteen rabbit bucks of the local population weighting between 3150 – 4000 aging from 5 to 12 months. Animals were the product of algerian local population gathered from different farms from the Tiaret region. Our work consider measurement of certain morphometric variables and appreciation of certain qualitative parameters

2.2. Measuring equipment:

- Metric tape for measuring parameters relating to lengths (except ear, paw and tail) and circumferences.

- Metal meter for measuring the length of the ear, paw and tail according to the recommendations of the FFC (1993).
- Scale for measuring live weight.

2.3. Parameters:

The study focused on the measurement of quantitative parameters and the appreciation of qualitative parameters. The quantitative parameters were lengths and widths as follows: body length (BL), head length (HL), total length (TL), distance between eyes (DE), lower back length (LBL), lower back width (LBW), ear length (EA), ear width (EW), leg length (LL); and circumferences: chest circumference (CC), turn of the forelimb (TF), as well as live weight (LW). The qualitative parameters are represented by the conformation of the body (including the wearing of the ears and the tail) and the color of the coat (dress).

2.4. Handling

For each rabbit we have registered the weight gain to determine live weight (in kg) with a commercial scale, precision $\pm 0.05g$. Rabbits were kept immobilized on a horizontal plane, we performed the measurement (in cm) after locating the landmarks on the surface of the animal's body (Table 05, Figure 4). These points made it possible to define the parameters appearing in table 06. In order to minimize measurement error, each measurement was taken twice (and even more) and the average was used in subsequent analyzes.

Point	Definition
A	Nasal apex (tip of the nose).
B	Occipital protuberance.
C	Ear base.
D	Apex of the auricular pinna (tip of the ear).
E	Atlanto-occipital joint.
F	Thoraco-lumbar joint.
G	Lumbosacral joint.
H	Last caudal vertebra.
I	Base of the tail.

J	Heel tip (os calcaneus).
K	End of the label.
L	A point of the diameter of the thorax.
M	Middle of the axes of the metacarpals.
N	Internal angle of the eye.

Table 05. The benchmarks used for the definition of the morphological parameters measured in the study

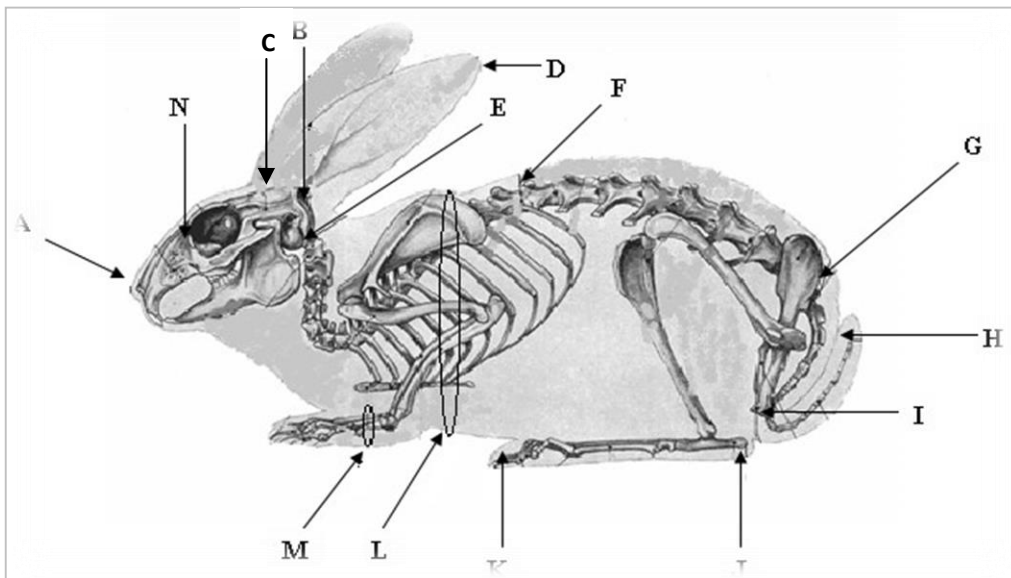


Figure 9. The landmarks used in the study to define the morphological parameters of lengths and circumferences in the local rabbit

Parameter	Definition
Body length	Measured on the midline of the body, between (E) and (I), while following the profile of the spine.
Length of head	Measured on the midline of the head, between (A) and (B).
Total length	Total length of the animal (tail excluded), measured between (A) and (I).
Distance between eyes	The distance between the internal angles of the eyes (N).
Lower back length	Measured between (F) and (G) while following the profile of the spine.
Lower back width	It is the average width of the lumbar region.
Chest circumference	Measured just behind the shoulders, the tape measure runs vertically behind the scapulae (L).
Turn of the forelimb	It is the diameter of the middle of the forelimb, measured at the level of (M).
Ear length	Measured between (C) and (D).
Ear width	Measured at the widest width of the ear (in the middle of the ear cartilage).
Leg length	Measured between (J) and (K).
Tail length	Measured between (I) and (H).

Table 06. Definition of measured parameters in the study

2.5. Statistics:

Data were collected and statistically analyzed using Microsoft Excel Spreadsheet Software (Microsoft Office Professional Plus 2013). The measured variables were weight and morphometric parameters.

3. Results and discussion

In order to assess some morphometric variables or quantitative parameters and the appreciation of certain qualitative parameters; Table 07 show all the measurements taken in this study.

N	Coat colour	weight (kg)	Measurements (cm)											
			BL	HL	TL	DE	LBL	LBW	LL	TL	EL	EW	CC	TF
1	Albino	3,45	43	11	56	6	17	15	9	9	12	6	31	7
2	Black	3,4	39	10	56	7	15	11	9	7	12	7	33	6
3	Albino	3,55	44	11	57	7	20	17	10	9	14	7	33	7
4	Black	3,6	35	12	58	6	15	16	9	7	13	7	35	7
5	Black	3,15	38	12	49	6	12	10	7	6	12	6	28	5
6	Sandy	3,9	39	13	59	6	15	18	9	7	14	6	36	7
7	Sandy	3,85	40	13	58	6	21	17	8	8	14	7	35	6
8	Sandy	4	45	13	60	7	22	19	10	9	15	7	36	8
9	Fawn	3,45	38	11	56	6	16	15	7	8	12	6	32	7
10	Fawn	3,5	39	11	56	6	16	16	7	8	12	7	33	7
11	black+white	3,85	40	9	49	6	12	18	10	9	13	7	36	6
12	black+white	3,9	40	11	55	7	15	19	10	8	15	7	34	8
13	light gray	3,45	35	12	54	6	14	14	9	8	12	6	36	7
14	black+white	3,35	41	13	56	6	15	11	10	9	13	6	36	7
15	Black	3.8	42	12	57	6	17	12	8	8	13	6	31	6
16	Black	3,4	40	12	55	6	16	11	9	7	12	7	34	7

Table 07. Phenotypic parameters of rabbit bucks

3.1. Quantitative parameters:

3.1.1. Weight:

The variation of weights within the number of rabbit bucks are recorded in Table 08.

N	Mean ± SD	CV (%)
N=16	3.75 ±0.25	7,01

Table 08. Weight variation of rabbit bucks

The results shown in Table 11 show the weight measured of 16 rabbit bucks of local Algerian population. It indicate that the average weight of our sample rabbit bucks is $(3.75 \pm 0.25 \text{ kg})$.

The weight registered has a moderate coefficient of variation with interindividual variability (7.01%). This result is close to those obtained by Shahin and Hassan (2000, 2002) on Red Baladi and Black Baladi breeds from Egypt and the White New Zealand breed; who concluded that the weight varies more than the majority of the morphometric variables measured. In the end, body dimensions are more reliable clues for determining body size than weight.

Breeding system where the farmer cannot control the feeding which is ad libitum; result less space and less mobility of rabbits (mobilization of body reserves). In the other hand; increasing the space allocated to animals allow locomotor activity and therefore slows growth (Combes and Lebas, 2003). Therefore, rabbits of close breeding are heavier than those of open breeding.

The breeds of rabbits are classified according to the adult weight, namely: the heavy or giant breeds (7-8 kg), followed by the medium breeds (3-4 kg), the light breeds (2-3 kg) and finally the dwarf breeds (order of 1 kg) (Chantry-Darmon, 2005), therefor, our rabbit bucks are classified as small breed.

3.1.2. Size:

Table 09 show the mean values and the coefficient of variation of the morphometric parameters relating to size body length (BL), head length (HL) and total length (TL) for rabbits bucks.

Parameter (Cm)	N	Mean± SD	CV (%)
BL	n=16	39.88 ±2.78	6.97
HL	n=16	11.63±1.15	9.87
TL	n=16	55.69±3.03	5.44

Table 09. Size variation within number of rabbit bucks (body, head and total lengths)

Based on the results in Table 09; the interindividual variability of body length (BL), head length (HL) and total length (TL) is slightly higher in rabbit bucks (CV% = 6.97%, 9.87%, 5.44%). These results can be justified by the completion of skeletal development by animals of class II (adults). According to Lebas (2002), the growth in length of the skeleton of the rabbit is terminated, therefore the size of the rabbit is fixed, around 140 to 150 days when the epiphyseal plates are "closed". Therefore, class II rabbits can be considered to have acquired the morphological characteristics of the breed and remain representative of the population.

3.1.3. General aspect:

3.1.3.1. Head:

N	Mean \pm SD	CV (%)
n=16	6.25 \pm 0.45	7.16

Table 10. Distance between eyes (DE) of rabbit bucks (cm)

According to Table 10, Our results are higher than those of Nezar with a DE=4.51 \pm 0,18. The large percentage of growth is the result of the increasing allometry of the bone skeleton which matures physiologically at an early age (Cantier *et al.*, 1969).

Ear size (Cm)	N	Mean \pm SD	CV (%)
EL	n=16	13 \pm 1.1	8.43
EW	n=16	6.56 \pm 0.51	7.81

Table 11. Size of the ears (ear length and ear width) of rabbit bucks

The results shown in Table 11 show no remarkable difference in ear length (EL) and width (EW) at rabbit bucks.

3.1.3.2. Trunk:

Tables 12 and 13 present the mean values \pm the standard deviations, and the coefficients of variation of the morphometric parameters relating to the format of the rabbit (CC, LBL and LBW).

N	Mean \pm SD	CV (%)
n=16	33.69 \pm 2.33	6.92

Table 12. Chest circumference (CC) of rabbit bucks (cm)

The previous results show that rabbit bucks have a slightly thick chest circumference. With an average circumference of 33.69 cm, the local adult rabbit appears to have a larger rib cage than those of other populations identified as light breeds such as the Tadla breed from Morocco (31cm) (Bouzerkaoui, 2002).

Parametre (Cm)	N	Mean \pm SD	CV (%)
LBL	(N=16)	16.13 \pm 2.83	17.52
LBW	(N=16)	14.94 \pm 3.09	20.67

Table 13. Length and width of the loins or lumbus (LB) of rabbit bucks

The results obtained in Table 13 show that the average size of LBL is equal to 16.13 \pm 2.83 and LBW of 14.94 \pm 3.09 with a high coefficient of variation (17.52% and 20.67% respectively).

The lumbar length (LBL) and lumbar width (LBW) of our rabbit bucks are higher to that of the local white Baladi rabbit from Egypt (Khalil, 2002) and (Afifi, 2002). According to Nezar (2007). The growth in length of the lumbar region is related to the growth of the lumbar vertebrae (growth of bone tissue); the latter is very early, on the other hand, its growth in width (filling of the loins) is the result of the development of other tissues, in addition to bone: muscle and fatty tissue. These two tissues have a development loan compared to the bone tissue, which reflects the average percentage of growth presented by the young rabbits for the width of the loins.

N	Mean±SD	CV (%)
(n=16)	7.94±0.93	11.7

Table 14. Tail length (TL) of rabbit bucks (cm)

The results shown in Table 14 show that the average tail length (TL) in rabbit bucks of algerian local population is equal to 7.94. The interindividual variability is considered in this type (CV of 11.7%).

The variability of this parameter is related to the variability of the number of coccygeal vertebrae. Barone (1973) declared that the number of coccygeal vertebrae is highly variable even within the same species, and the tail is more prone than other regions of the spine to individual, racial and specific variations. It is for this reason that it is eliminated in the measurement of the size of the animal.

3.1.3.3. Limbs:

Table 15 presents the mean values and the coefficient of variation of the measurements carried out on the turn of the forelimb (TF) and leg length (LL)

Parameter (cm)	N	Mean ± SD	CV (%)
TF	(n=16)	6.75 ±0.77	11.48

LL	(n=16)	8.81±1.11	12.58
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Table 15. Forelimb circumference and length of rabbit bucks

Our findings show a higher TF (6.75 cm) in comparison with 5.31 ± 0.53 of Algerian local population announced by Nezar N. (2007).

This can be explained by an important bone growth in thickness. This is because animals that have less space (cage or fattening room) tend to develop thick and short limbs. Contrariwise, free animals move more; which promotes the development of limbs in length.

Rabbit bucks presented an important interindividual variability ($CV\% = 11,48\%$), this result testifies to the presence of a degree of heterogeneity in the sample for this parameter and the presence of individuals with turn of the forelimb thin than others.

3.2. Qualitative parameters:

3.2.1. Coat colour:

According to the results shown in Table 16, the most common coat of the local rabbit bucks is single colour (81.25%). The compound colored coat represent only 18.75% of the sample studied. All the rabbits with a compound coat have a two-tone coat: a white coat with a color pattern (black); this coat is of ordinary type.

Coat colour	%	Couleurs	Rabbit bucks	%
Single colour	81.25	Black	05	31.25
		Sandy	03	18.75
		Albino	02	12.5
		Fawn	02	12.5
		Light gray	01	6.25
Compound (02 colours)	18.75	Black + wight	03	18.75

Table 16. Distribution of rabbits according to coat colour

The distribution of the rabbits examined according to the color of the coat shows a predominance of the black colour with (31.25%) and sandy with (18.75%), black and wight equal to (18.75) followed by albino and fawn with (12.5%) for both. These results are in agreement with those reported for the rabbit from the Kabyle population, the precise distinction of which is sometimes made difficult by the multitude of colors (Berchiche and Kadi, 2002; Djellal, Mouhous and Kadi, 2006).

4. Conclusion

Local rabbit bucks are characterized by a light average adult weight allowing the population to be classified in the category of small rabbit breeds.

We recommend to continue this study by increasing the number of rabbits and reaching other regions; in order to enrich the database on the local rabbit.

**GENERAL
CONCLUSION
AND
PERSPECTIVES**

General conclusion and perspectives

The work carried out during this study made it possible to assess a reproductive and morphometric characterization of rabbit bucks from the local population reared in the Tiaret region under a semi-arid environment by evaluating the reproductive performances and appraising morphological parameters.

In the first part, we have study the effect of rabbit bucks age on the quality of sperm by analyzing the volume, pH, concentration, motility, vitality and abnormal spermatozoa. We found that rabbit bucks of local population represent significant changes of sperm characteristics according to their age. Concurrently, we have used them in artificial insemination and it remain good with agreeable results.

In the second part and on the same axis of reproductive characterization we determined the parameters influencing reproduction in males by analyzing the sperm under different temperatures.

The two first parts of the study allowed us to observe that:

- During the summer season the Libido decreases, the pH of the sperm and the morphological changes of the sperm increase.
- Motility on average was higher in bucks kept under high temperatures comparably with low temperatures ($\leq 15^{\circ}\text{C}$).
- The rabbit of the Algerian local population good and desirable for reproduction (natural breeding and artificial insemination) in comparison with other strains although they are raised under severe conditions.
- During high temperatures, which occurs in Algeria in summer, reaction time decreased slightly, the decrease of testosterone, semen pH and sperm morphological alterations increased.
- It seems that the males of the local population of 10 month old rabbits are the best breeders and desirable.

Finally, and in order to evaluate morphometrics we appraised the measurements of some parameters of bucks from the same population. This part conducted us to notice that:

- The local rabbit is characterized by a light average adult weight allowing the population to be classified in the category of small rabbit breeds.

The results of our work provide interesting prospects for improvement and completion in local rabbit breeding. Indeed, further investigations are needed, including the study of genetic traits of our breed to assess a complete characterization of our local rabbit breeds.

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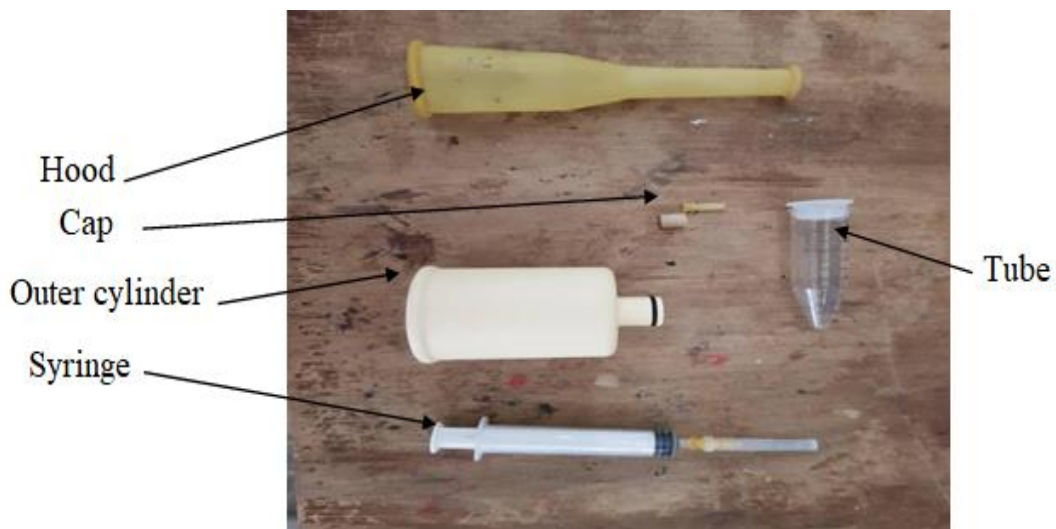
ANNEXE

Diet

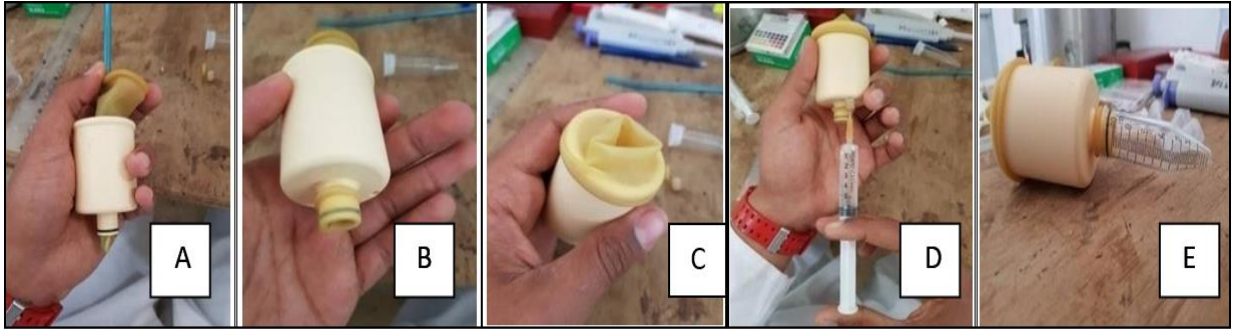
The animal feed was provided with a commercial feeds (A standard feed (Table 4) while the watering was provided by automatic waterers *ad libitum*.

Composition	%
Soft wheat	10
Soybean meal	12
Carbonate Calcium	2
Dicalcium phosphate	0,8
Grain corn	47,2
Soft wheat bran	26
micotoxin sensor	0,05
Salt	1
CMV	1
chemical composition	%
MS	86,63
PB	13,81
MG	2,93
Digestible energy (Kcal)	2820
Crude Cellulose	4,34
NDF	17,7
ADF	5,54
ADL	2,31
Starch	41,11

Standard feed composition



Different parts of artificial vagina (original photo, 2018)



Different steps to prepare artificial vagina (Original photo, 2018)



Semen collection techniques in rabbit bucks according to Lebas (2010)

PUBLICATION

SPERM QUALITY IN RESPONSE TO AGE IN LOCAL RABBITS REARED IN SEMI-ARID ENVIRONMENT (TIARET, ALGERIA)

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Abstract. The aim of this study was to evaluate the age effect on quantity and quality of rabbit semen raised in semi-arid environment of Tiaret region. The study was conducted at the experimental farm of Ibn Khaldoun university of Tiaret. A total of 20 rabbit bucks of the local Algerian population (5-11 months of age) weighting between 3010g and 4540g were collected randomly and exposed to an extensive rhythm. The average value of libido was 25,17±20,94 seconds (sec.). The ejaculate volume was 1,48±0,33ml and the pH 7,67±0,36 for bucks of 11 months of age. The analyses of semen show no significant for mass and individual motility (6,84±1,70 and 2,96±1,04 respectively). The rate of vitality was 61,18±18. However, the age of bucks significantly affected the concentration and abnormal spermatozoa ($p<0,05$). In this study, most of semen parameters were influenced by the age and rabbit bucks of the Algerian local population seems desirable for reproduction in compare with other strains.

Keywords: Rabbit, fertility, age, semen, spermogram.

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1. Introduction

The best rabbit's performances use depends entirely on good reproduction management. According to Alvarino (2000); male is the basis of breeding success playing a key role in the achievement and profitability of the rabbit's breeding. The buck in natural breeding is used to fertilize 8 to 11 does (Roca, 1994; Osechas & Becerra, 2006) in contrast by artificial insemination it can be used to fertilize 100 does at a time, because of the fertilizing capacity of semen (Eid, 2008).

Semen evaluation traits informs about the spermatozoa fertilizing capability (Boiti *et al.*, 2005). Moreover, there are a wide variety in semen traits, and different factors such as the collection frequency, lighting programs, buck age, might influence qualitative and quantitative sperm production (Boiti *et al.*, 2005). To obtain an optimal quantity of sperm and spermatozoa, it is necessary to define the conditions of use of the bucks (Boulbina *et al.*, 2012). The analyses of sperm production is significantly highly correlated with sexual activity (Benia *et al.*, 2018), it is necessary to identify the puberty age, sexual maturity, the response to the collect and factors affecting the sperm production. In order to characterize male reproductive performance of the Algerian local rabbit population; the aim of this study was to assess the sperm quality evolution with age in local rabbits raised in the semi-arid environment.

2. Materials and methods

The study was conducted at the experimental farm of Ibn Khaldoun University of Tiaret (western Algeria) during 2019. Rabbits were the product of a crossing between local populations up to the 5th generation. Twenty rabbit bucks of the local population weighting between 3010 – 4540 were collected (from the 5th to the 11th month of age) to evaluate the semen quantity and quality evolution. Animals were housed individually in wire cages arranged in flat-deck layout on one level. Ventilation and lighting were naturally provided. Automatic waterers were used, and the rabbits were fed *ad libitum* with granulated commercial diet (13,81% crud protein, 2820 kcal digestible energy/kg).

The semen collection was made by using an artificial vagina, using a teaser doe. Libido was recorded in terms of reaction time in seconds and was estimated from the time the doe was placed inside the buck's cage up to the point when the buck started to mount the doe (Daader *et al.*, 1999). After collection, the volume of the ejaculate was assessed by reading the graduation of the collecting tube. Sperm volume is deducted after removal of the gel fraction. The collection tube was immediately put into an electric oven at 37 °C. The pH of the semen is determined by a pH paper. Then, the mass (MM) and individual (IM) motility of the spermatozoa were determined under a phase contrast microscope. Mass motility was appreciated by placing a drop of pure sperm between slide and lamella observed at magnification (x10), a note from 0 to 9 was attributed to the movement of sperm mass observed on the Petitjean scale (1965) mentioned by (Boussit, 1989). Individual motility was assessed after dilution of the sperm with a commercial diluent at the rate of 1/5 and 4/5 diluter volumes. A drop of diluted semen was observed between slide and lamella at magnification (x40), a note from 0 to 4 was attributed to the individual movement of spermatozoa observed on the Adrieu scale (1974) according to (Boussit, 1989).

The concentration (C) in spermatozoa ($10^6/\text{ml}$) was determined using a malassez cell from a drop of seed diluted to 1/200 with the diluter. Counting was performed under the microscope at magnification (x40) (Boussit, 1989).

The vitality was determined by the preparation of a smear using eosin-nigrosine vital staining, a drop of semen was mixed with a drop of the dye, and then the mixture was gently spread along the blade. The smear was left for a few seconds, then it was observed under magnification microscope (x100). Dead sperms spread the dye through their damaged membrane, while living spermatozoa with their functional membranes do not diffuse the dye and therefore remain colorless. A random count of 150 spermatozoa was performed along the smear, from which dead spermatozoa were distinguished from the living (Boussit, 1989).

The percentage of abnormal spermatozoa (AS %) was studied on the same sample of the stained smear. 150 spermatozoa were randomly counted and abnormal spermatozoa were distinguished (Boussit, 1989).

Data were collected and statistically analyzed using one-way ANOVA (IBM® SPSS 25 software). The variables analyzed were macroscopic sperm parameters (weight, Libido, volume, and pH) and microscopic parameters (mass motility, individual motility, concentration, vitality, and percentage of abnormal sperm) and the effect of age on these parameters.

3. Results and discussion

In this work, a total of 103 semen samples from the 5th month until the 11th age were collected and analyzed Table 1.

Table 1. Mean±SD values of the semen parameters analyzed

Age (Months)	5 (n=10)	6(n=15)	7(n=16)	8(n=17)	9(n=16)	10(n=15)	11(n=14)	Total (n=103)
weight (g)	3557±367,9	3675±332,7	3745±336,9	3858±354,5	3955±304,1	3814±287,5	3736±354,3	3777±331,2
Libido (s)	33,7±16,29	26±19,01	23,38±27,29	34,82±26,97	24,56±19,83	16,27±12,80	19,14±12,98	25,17±20,94
pH	7,13±0,26	7,10±0,20	7,52±0,30	7,61±0,50	7,38±0,30	7,59±0,34	7,67±0,36**	7,44±0,39
Volume (ml)	0,98±0,45	1,48±0,33*	1,44±0,33	1,12±0,59	1,03±0,36	1,05±0,28	0,99±0,27	1,17±0,43
Concentration (10 ⁶ /ml)	314±52,5	412±86,5	405±94,5	426±158,4	456±175,2	548±151,2	599±148,9*	456±154,4
Mass motility	6,5±0,71	6,27±0,80	6,38±1,36	6,59±2,45	7,19±2,04	8,07±1,10	6,86±1,79	6,84±1,70
Individual motility	2,8±1,03	3,13±0,64	2,88±1,20	2,41±1,18	3,19±1,17	3,4±0,74	2,93±1	2,96±1,04
Abnormal sperms (%)	46,5±6,54	41,13±6,66	38,25±8,06	29,29±12,67	30,69±7,58	39,33±8,06	47±3,40*	38,17±10,21
Vitality (%)	53,05±18,1	46,53±8,9	58,94±10,4	60,71±23,5	66,5±21,1	75,87±9,7	64±14,7	61,18±18

*Refers to a significant difference ($p < 0,05$). **Refers to a significant difference ($p < 0,005$).

In our work, the average value of libido was 25,17±20,93, lower than 14,5 and 21,9 respectively reported for the Black Baladi and White New Zealandies rabbits by Safaa et al. (2008). These differences appear to be related to the genetic origin of the rabbits and the breeding program to which they have been subjected (Lankri *et al.*, 2019). While the pH of semen were significantly higher ($p < 0,05$) at 11th month of age with 7,67±0,36, this can be due to the increased secretions of the vesicular glands (Rigal, 2008).

The ejaculate volume of the rabbits' semen, in this work was about 1,17±0,43 ml, higher than values from 0,3 to 1 ml reported by Orgebin-Crist (1968), Cole and Copps (1977) and Lebas et al. (1996) in adult rabbit sperm, and Boulbina et al. (2011) with 0,86 ml on the local rabbit population. Feeding *Ad libitum* increase the libido and the volume of the sperm according to (Alvarino, 2000). In the other hand; the volume of the semen, gel fraction, sperm motility, sperm concentration and morphological alterations, show high variations among the different breeds (Dubiel *et al.*, 1985; Abo El-Ezz *et al.*, 1985). The highest semen volume was recorded at six months of age which coincided with March and according to Boussit (1989) the volume of ejaculate reaches its high values from March to June.

Joly and Theau-Clément (2000) reported that the biological characteristics of the semen (volume, concentration, motility, morphological alterations ...) are very variable between and intra-breeds, but on average the values of these parameters increase with the age of bucks (from 5 months to 24 months). In this work, the adult's semen concentration was significantly higher with 599±148,94x10⁶/ml. According to Theau-Clement et al. (2003) the age of males influences significantly the sperm concentration and production. Our results are higher than 232 x10⁶/ml and 220 x10⁶/ml reported by Safaa et al. (2008b) of two selected lines of New Zealand rabbit bucks, otherwise, it was recommended to make a collect one a week (Tacke *et al.*, 1995; Bencheikh 1993; 1995), however, our rabbits were collected 2 to 3 times in a month. The different studies were

based on observation and counting, which are probably an additional source of variability in the results (Cabannes, 2008).

Sperm motility is a very important parameter that reflects the quality and has a significant effect on egg cell fertilization (Wysokińska *et al.*, 2013). The higher rate of motility in this work was recorded in the 10th month of age. However, the average of total values of mass and individual motility were about 6,84 and 2,96 respectively, lower than values reported by Boulbina et al (2011) in male rabbits of the local population with a mass motility of 7,68 and an individual motility of 3,57. It was estimated that semen had a good mobility with mass motility with an appearance of waves ($\geq 6/9$) and individual motility with a fast progression ($\geq 3 / 4$) (Boussit, 1989).

The vitality rate of the sperm collected increased with age and the highest value was recorded at 10th months of age with 75,87±9,69%. According to Boulbina et al (2012) delayed puberty gives more time for testicular and epididymal function to settle, and consequently a higher quality semen from the first ejaculates. Moreover it start decreasing after the 10th month because semen quality generally decreases in older rabbit bucks (Castellini, 2008).

The total sperm abnormalities rate was significantly higher at 11th months with a value of 47±3,40%, it was explained by the high environmental temperatures recorded at the moment of collection, because the age of 11 months coincided with August. Which was August characterized in the region, these results showed that temperature changes that correspond to the month of August. However, a significant interaction was observed between the breed and season (Safaa *et al.*, 2008a).

4. Conclusion

It could be concluded from the current study that the age has a huge effect on semen production and characteristics.

The Algerian local bucks remain good and desirable for reproduction (natural breeding and artificial insemination) in compare with other strains like New Zealand rabbit bucks although it raised in harsh environment.

It appears that rabbit local population bucks of 10th month of age are the best reproductive and desirable.

We recommend to improve livestock under controlled conditions to have better results.

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