PHD THESIS SUMMARY

Keywords: videomorphometric, area, length, width, perimeter, ellipticity, elongation, roughness and regularity

Animal husbandry is one of the oldest human occupations, and rearing sheep was practiced very early in the history of human development, with domestication of sheep ancestors today. Thus, the oldest archaeological traces relating to domestication of sheep have been found in Iraq, they dated from the VIIth Millennium BC, the data demonstrates the importance and role of this species in the development of modern human being.

Current paper, entitled VIDEO-*MORPHOMETRIC RESEARCHES REGARDING RAM SPERM*, reads methodological norms in force and contains two major parts:

Part I, represented by a literature review contains a number of four chapters entitled:

THE IMPORTANCE, GUIDELINES AND WORLD SHEEP REARING PERSPECTIVE, REPRODUCTION IN RAM (*OVIS ARIES*), ASSISTED REPRODUCTION TECHNIQUES IN SHEEP AND SPERM ASSESSMENT FOR ARTIFICIAL INSEMINATION.

Part II, consists of personal research and contains four chapters:

THE AIM OF THE RESEARCHES, EXPERIMENTAL LOCATION, BIOLOGIC MATERIAL AND WORKING PROTOCOL, RESULTS REGARDING THE MACROSCOPIC EXAM OF SPERM, RESULTS REGARDING THE USUAL MICROSCOPIC EXAM, RESULTS REGARDING MORPHOMETRIC EXAM OF SPERM.

The Doctoral Thesis ends with CONCLUSIONS AND RECOMMENDATIONS, and a list of REFERENCES.

The study was conducted on the I.C.D.C.O.C. Palas Constantá (INSTITUTE OF RESEARCH AND DEVELOPMENT FOR SHEEP AND GOATS BREEDING), Romania and the Laboratory of Animal Reproduction, Animal Sciences Faculty, University of Agronomical Sciences and Veterinary Medicine, Bucharest, Romania. All animal care and procedures used in the study were performed in such a manner that it stipulated in the working protocols without affecting environment and animals welfare.

A total of 20 Merino rams (*Ovis aries*) aged 2 to 6 years were used in the study. The rams are part of a research flock established and maintained at the I.C.D.C.O.C. Palas Constantá farm. The rams were maintained under uniform nutritional conditions, and to avoid energy partitioning towards wool growth between individual rams, all animals were shorn approximately three months prior to the onset of semen collection.

The animals used in this study originated from the same base population. All rams were maintained according to ethically approved husbandry practices, and under uniform nutritional conditions. All the rams were alloted to three experimental groups, depending on their age: 6 young males (younger than three years old), 9 adults (3-4 years old) and 5 old males (over four years old).

The studied rams were Merino Palas breed, one of the native Romanian breed.

In Chapter I there are described, based on specialized literature, THE IMPORTANCE, GUIDELINES AND WORLD SHEEP REARING PERSPECTIVE. There are presented aspects regarding the spread of sheep across the globe, some data about the global agricultural production in the sheep field, the current global sheep population at more than 1 billion head with 19 per cent found in Asia and Africa and data from Food and Agriculture Organisation . It was illustrated the **World Sheep Inventory - Top 10 countries, The evolution of the worldwide sheep population – thousands heads,** the percentage distribution of the flock of sheep by continents, data about sheep milk and meat production and the trend in sheep rearing.

Chapter II, called REPRODUCTION IN RAM (*OVIS ARIES*) presented aspects of Reproductive anatomical peculiarities of the testicle, the penis and genital physiological peculiarities in ram, represented by describing the process of spermatogenesis. There are explained the mechanisms of sperm ejaculation, sexual reflexes in ram, semen collection and environmental factors action over it. The factors that may affect sperm quality are considered: temperature, light, environmental response, osmotic pressure, microbial flora, nutrition and chemical attack. There are explained the spermatozoon morphology and the hormonal regulation of spermatogenesis.

In Chapter III, entitled ASSISTED REPRODUCTION TECHNIQUES IN SHEEP, it was presented the potential of assisted reproduction techniques to assist sheep producers to optimize the production and reproductive efficiency of their flocks. The aspects about the application of

assisted reproduction techniques, such as artificial insemination (AI) and multiple ovulation and embryo-transfer (MOET) are explained and also gives arguments which allow sheep farmers to produce lamb and muttons more cost-efficiently. Assisted reproductive techniques (ART's) such as artificial insemination (AI) and *in vitro* embryo production and transfer (IVEP) can then contribute significantly, if applied properly, to optimize and ensure cost-efficient livestock production (Byrne *et al.*, 2012). Within subchapter 3.1 there are data about artificial insemination in sheep- advantages and disadvantages, followed by multiple ovulation and embryo transfer explanations in subchapter 3.2., and also in subchapter 3.3. the sexing of sperm by DNA.
CHAPTER IV presents very detailed the SPERM ASSESSMENT FOR ARTFICIAL INSEMINATION. There are presented the two methods of sperm collection, the AV method and EEJ method and also the possibility of Collection of the epididymal sperm. It was going on with sperm assessment by macroscopic, microscopic and morphometric methods, and also the possibility of CRYOPRESERVATION OF SEMEN. The chapter IV ends with previous researches regarding factors affecting sperm quality.

Part II, the original research, starts with explanations regarding the aim of the researches, the experimental location, the biologic material and the working protocol, all of these in CHAPTER V. There were included data and photos about the biologic material and the working protocol was detailed.

The CHAPTER VI, called RESULTS REGARDING THE MACROSCOPIC EXAM OF SPERM gives detailed original results about the semen traits evaluated by the macroscopic exam. The m**acroscopic exam** was made by raw semen, most of the features being assessed in the collector glass. The assessed macroscopic parameters were the volume of the ejaculate, the sperm colour and smell, the semen consistency and pH and the wave motion of sperm.
CHAPTER VII, entitled RESULTS REGARDING THE USUAL MICROSCOPIC EXAM included also, original results and comparisons with other researches in the field. The microscopic examination covers the following characteristics: density of sperm; sperm concentration; sperm mobility and morphological examination of the sperm, including the percentage of the normal and abnormal live spermatozoa.
CHAPTER VIII, entitled RESULTS REGARDING MORPHOMETRIC EXAM OF SPERM, represents the main part of the present research. It was represented by the video – morphometric

research upon the semen material from the 20 studied rams. There were carried out morphometric determinations with the aid of Full ISAS CASA System, composed by ISAS software, UB203i phase contrast microscope with epi-fluorescence and heated stage, heated stage controller, camera and computer was used for sperm morphometry. With the aid of ISAS v1 in our study, the morphometric features of sperm cells were performed from stained samples. The data were recorded at the Faculty of Animal Science, U.A.S.V.M. Bucharest. ISASv1 Morphometry gave the following morphometric parameters: **head size or primary parameters** (area, length, width and perimeter), **head shape or derived parameters** (ellipticity, elongation, roughness, regularity). The measured morphometric parameters are summarized in tables and charts and the statistical analyze was done.

CAPITOLUL IX, CONCLUSIONS AND RECOMMENDATIONS, ended the doctoral thesis. All the sperm morphologic and morphometric parameters obtained throughout the study showed considerable variation (the magnitude of the variation for each trait being included).

The values of the **ejaculate volume** ranged between 1.4096$\pm$0.081753 mL and 1.8224$\pm$0.067367 mL. The lowest value of the volume of ejaculate was recorded in the group of males older than 4 years old (1. 59312$\pm$0.022755348 mL), meanwhile, the highest value was recorded in the group of 3-4 years old rams, (1.729688889$\pm$0.026386997 mL). No significant differences were obtained for ejaculate volume between samples of the youngest rams in the study beside the oldest rams, but very significant difference between the others age category, rams 3-4 years old – younger than 3 years old and also rams 3-4 years old - rams older than 4 years old. Depending on the collection method, the mean values of the semen volume were higher in the case of artificial vagina method compared to electro-ejaculation method. There were recorded very significant differences between the samples obtained by the two collection methods, artificial vagina and electroejaculation.

The **colour of semen** was normal, white to yellowish, in all the analysed ejaculates.In close correlation with the colour it was the **smell of sperm**, in all cases a smell of protein, almost fresh egg.

The **consistency of semen** recorded values varied from 3.32 to 3.72, on the scale from 0 to 5.The mean values of the consistency of semen depending on rams age was the highest in adult rams, followed by the younger rams, under 3 years old, the lowest value being recorded in the oldest rams. The mean value of the semen consistency depending on the collection method revealed the

fact that the semen consistency was higher in the case of using the artificial vagina and lower in the case of using the electroejaculator. The lowest value of the **semen pH** was 6.8488±0.012715 and the highest one, 7.1672±0.028528. The lowest value of this parameter was recorded in adult rams. Higher values were recorded in the category of older rams, and the youngest rams in the study recorded the value of 7.098267±0.010749. There were recorded small differences between the two techniques of sperm collection.

The semen **wave motion** depending on rams' age ranged between 3.6±0.048639 in the category of the oldest rams and 3.648889±0.052118 in the category of adult rams. We could conclude the superiority of the results obtained by artificial vagina collection method, beside the elecroejaculation collection method.

The values of the **sperm motility** ranged between 80.6±1.235584% and 90.4±0.369685%. The highest value of sperm motility was recorded in the 3-4 years old rams, meanwhile, a lower value of sperm motility was recorded in the category of the oldest rams. The difference between the two collection methods represents only 0.59%, in favour of the artificial vagina method.

The mean values of **semen concentration** ranged between 1.1224±0.029128 $x10^9$ sperm cells/mL and 2.8412±0.029031 $x10^9$ sperm cells/mL. The highest value of sperm concentration was recorded in 3-4 years old rams category. The semen concentration in sperm cells was 2.1178±0.0033516 $x10^9$ sperm cells/mL for the AV method and 2.102828±0.057555 $x10^9$ sperm cells/mL for the EEJ method. Very significant differences were obtained for semen concentration between samples of different age rams.

The highest recorded value of the morphologic **normally sperm cells** ranged between 91.96±0.329039 % and 85.44±0.583324 % with the highest value of this parameter recorded in the adult ram category. Taking into consideration the collection technique, the morphologic normally sperm cells values were higher in the case of EEJ method, comparing the AV method, even the two values did not differ significantly.

The highest mean value of the morphologic **abnormally sperm cell**, was 14.96±0.717589 % and the lowest value 8.04±0.329039%. Depending on ram age, this parameter recorded the lowest value in the case of 3-4 years old rams. There were counted more abnormal sperm cells in case of AV than in case of EEJ.

The mean values of **the length of the sperm head** ranged between 7.4276±0.029537 μm and 7.9064±0.016237 μm. The lowest value was recorded in older than four years ram category,

followed by the adult ram category. Very significant differences were obtained for sperm head length between samples of the youngest rams in the study beside the oldest rams, and also between 3-4 years old rams – and the oldest rams too,. There were not recorded differences between the 3-4 years old rams and younger than three years old rams. Little superiority of the length of the sperm head in favour of the electroejaculation collection method, beside the artificial vagina collection method was noticed. There were not recorded differences between the data obtained by grouping the samples upon the collection method, the AV method and the EEJ method. The lowest mean value of the **width of the sperm head** was recorded in the younger category, 4.3078±0.009706 μm, followed by the category of the oldest rams, with a mean value of 4.51232±0.004808 μm. The highest mean value was calculated in the adult rams, 3-4 years old, as 4.609556±0.007886 μm. Very significant differences were obtained for sperm head width between samples of sperm related to the age of the ram,but there were not recorded differences between the collection methods.

The superiority of the adult rams regarding the **area of the sperm head** beside the younger and also the older age category is concluded, with very significant differences. The mean value of the area of the sperm head was higher in case of electroejaculation method, than the one reported in case of artificial vagina collection method.

The values of **the sperm head perimeter** ranged between 17.04829±0.065817 μm and 19.26479±0.031957 μm. The highest mean value of the perimeter of sperm head was recorded in adult rams' category, followed by the category of older than 4 years old rams. Analyzing the values of the sperm head perimeter in relation to the collection method, it may notice that the values reported from the measurement of the sperm head obtained by electroejaculation method were higher than the ones obtained by artificial vagina method.

There could be concluding that the recorded **head ellipticity values** ranged from 1.635451±0.00757 to 1.817234±0.008555. The ellipticity of the sperm head recorded different values in the three age categories, with significant differences. The higher values of ellipticity indicated the sperm heads of the young rams are thinner. Sperm with more elliptic heads swim faster. The mean values of the ellipticity of the sperm head depending on the collecting method of semen recorded small differences between the values.

The head elongation index varied throughout the current study between 0.240967±0.002164 and 0.289929±0.002122. Thus, it may conclude that the highest value of the elongation index

was recorded in the youngest category. As the head elongation index is closer to zero, the rounder the head sperm is. The mean value of the elongation of the sperm head in case of artificial vagina use was 0.259293±0.00092 and the mean value of the elongation of the sperm head in case of electroejaculation use was 0.257642±0.001452, the difference represents only 1%, this value brought non significant differences between the AV method and the EEJ method.

The mean values of **the head roughness** ranged between 1.267035±0.001221 and 1.448358±0.001012. The use of artificial vagina gave the sperm cell a lower value of roughness index than the use of electroejaculation. The value of this index is lower, the sperm head are more amorphous or irregular.

T**he sperm head regularity** ranges from 0.743154±0.000006 to 0.788751±0.0000826. Considering the age of ram, our study reveals the fact that the highest value of the head regularity was recorded in the youngest age category. Considering as criterion the collection method, in the case of using the electroejaculation method the recorded values are superior than in case of using the artificial vagina method. Very significant differences were obtained for sperm head regularity between samples of sperm related to the age of the ram.

Microscopic evaluation showed that most parameters did not differ significantly between sperm obtained from different age ram ejaculates, excepting ejaculate volume, between the adult rams and the other two categories, and also between the two collection methods. Macroscopic evaluation showed that most parameters did not differ significantly between sperm obtained from different age ram ejaculates, excepting semen concentration. Morphometric analysis indicated significant differences between the age categories for the head width, area, perimeter, and index parameters as: ellipticity and elongation and regularity. The lack of significant differences indicated that other factors may affect the reproduction efficiency difference observed between the two methods. The results obtained for this part of the overall study emphasizes further research to correlate morphometric traits of ram sperm with conception rate of ewes to determine whether morphometric traits need to be included in sperm quality tests for ART's.

Overall, the morphometric parameters measured on fresh ejaculated ram sperm in this study are consistent with that found by several other researchers for head length, -area, -perimeter, ellipticity, elongation, roughness, and regularity (Martí *et al.*, 2011; Maroto-Morales *et al.*, 2012; Yániz *et al.*, 2012, Boshoff, 2014).