

ABSTRACT

Growth and sheep exploitation has been and remains an important objective, because this species can use as food less valued feedstuff, and the need for housing and care are less expensive than in other species.

Assisted breeding, shorter calving interval, heat deseasonalisation and accelerated calving through hormonal and non-hormonal methods can successfully contribute to the development of two births per year, three births in two years and increase prolificacy in sheep.

Success of biotechnologies application will only be possible there where is a concern supported by proper application of all technical and biotechnological steps. This can result in remarkable results in increasing fertility and prolificacy in sheep and obtain progenies with higher morphological productivity, to justify the breeder effort and the diffusion of knowledge on introducing and extending biotechnological breeding methods.

The problem of reproduction intensification in sheep breeding by reducing the interval between births, is a concern both nationally and globally, as I consider that this theme is current.

Present paper entitled, RESEARCHES ON PARAMETERS DYNAMICS IN SHEEP BREEDING IN TERMS OF APPLIED BIOTECHNOLOGY, reads methodological norms in force and contains two major parts:

Part I, represented by bibliographic research contains a number of five chapters entitled:

GENITAL APPARATUS MORPHO-PHYSIOLOGY IN RAM, EVALUATION OF SPERM PRODUCTION IN RAMS, MORPHO-PHYSIOLOGICAL PARTICULARITIES OF REPRODUCTIVE FUNCTION IN SHEEPS, FACTORS AFFECTING REPRODUCTION ACTIVITY, PARTICULARITIES OF SEXUAL CYCLE.

Part II, consists of personal research and contains four chapters: NATURAL DYNAMICS OF BREEDING PARAMETERS IN SHEEP, ASSESSEMENT OF SEMEN INDICES FROM RAMS DURING PRIMARY AND SECONDARY BREEDING SEASON, QUALITATIVE ASPECTS OF RAM SEMEN WITH EN EYE TO FREEZING, REPRODUCTIVE PARAMETERS IN SHEEP, OBTAINED BY OESTRUS SYNCHRONIZATION BIOTECHNOLOGIES.

Research for this thesis were conducted in sheep farms located in the north of Moldova. Some determinations that required special equipment and high workload were

conducted in collaboration with the Laboratory of Breeding and Biotechnology of ICPCOC Palas Constanța, with Electronic Microscopy Laboratory staff of the University "Ovidius" Constanța, and with Reproduction departament staff from the Faculty of Veterinary Medicine of Iasi.

The thesis ends with general conclusions, a list of appendices and bibliography.

In Chapter I are described, based on specialized literature, GENITAL APPARATUS MORPHO-PHYSIOLOGY IN RAM, and presented aspects of Reproductive anatomical peculiarities of the testicle, the penis and genital physiological peculiarities in ram, represented by describing the process of spermatogenesis. Below, are 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.

Chapter II, called EVALUATION OF SPERM PRODUCTION IN RAMS. A male fertility depends on semen's quality and quantity. The notion of quality of semen covers macro and microscopic characteristics of ejaculate, sperm fertility related. To assess the quantity and quality of semen are used macroscopic, microscopic, biochemical and bacteriological laboratory methods. Assessment is carried out immediately after collection.

Macroscopic examination appreciate: ejaculate volume, semen color, odor, density and the presence of sperm waves. Microscopic examination of semen, provides more reliable data regarding the ejaculate quality it is able to evaluate: sperm concentration, sperm motility, percentage of viable, teratologic or immature spermatozoa, etc.

In Chapter III are explained, MORPHO-PHYSIOLOGICAL PARTICULARITIES OF REPRODUCTIVE FUNCTION IN SHEEPS. Female genital serves to develop female gametes (eggs), secrete a number of hormones indispensable for reproductive function and is the center of some processes as: gametes migration, fecundation, implantation, product development design and performs all phenomenons necessary for calving and fetal annexes expulsion.

In the first subchapter is described the genital morphology in sheep. Is composed of: female gonad (ovary) and genital tract (oviduct, uterus, vagina and vulva). The sheep genitals generally resembles the cow. Ovaries have a length of 1.5 - 2.5 cm and are easily flattened. Uterine horns of the same orientation as the cow, being proportionately reduced. Uterine body has a length of 2 cm and cervix of 2 to 3 cm. Abundant flower has two folds, the upper being more developed than the lower, covering as a flap the inner lumen. Uterine caruncles are in

number from 88 to 96 and have a slightly concave surface. The vagina has a length of 6-8 cm and vaginal vestibule of 2 to 5 cm. Does not have Gärtner channels.

Genital physiology in sheep is represented by the process of oogenesis and folliculogenesis. Functionality of female genitalia is characterized by a rhythm that begins at puberty and ends at climacterium. Sexual cycle represents all gametogenic and endocrine changes, through which are released one or more eggs suitable for fertilization.

In Chapter IV, are presented FACTORS AFFECTING REPRODUCTION ACTIVITY.

Puberty and sexual precocity in sheep. Puberty is defined as the age at which reproduction is possible in females, the time being determined by the appearance of the first heat. As in other species, sexual maturity is dependent on the state of maintenance, nutrition, precocity rate, over which is superimposed influence of natural climatic factors in different seasons. Maturation of ovarian follicles. At birth, lamb ovaries contain all the eggs needed for reproductive life. Thus, the ovary contains tens of thousands of oogonia, but of them most degenerate and disappear during prepubertal stage. Maturation and growth of primary follicles are continuous processes and occur at regular intervals of 14-17 days. Sexual season in sheep is placed in late summer and early autumn, and its duration is variable from 80 to 150 days. Are also presented genetic variability issues, nutrition, the effect of light on reproduction, age and state of maintenance and exogenous hormones.

In Chapter V, are described PARTICULARITIES OF SEXUAL CYCLE. Maximum intensity in sheep breeding is in late summer and early autumn, when the length of the "daylight" decreases. The rest of the year, end of winter and spring, the ovary is at rest, with very low activity. It is clearly delimited a period of sexual activity called "sexual season" and a rest period called "seasonal anoestrus." After calving heats may not appear for a while. This sexual rest is called postpartum (puerperal) anoestrus or lactation anoestrus. All periodic and functional changes of the genital apparatus, accompanied by mental changes are known as heat, and all morphological and physiological changes that occur between two periods of heat is the sexual or gametogen cycle.

Peculiarities of the sexual cycle in sheep are supplemented by notions of neuro-endocrine regulation, the occurrence of oestrus and time of ovulation, factors that condition ovulation and artificial insemination.

Chapter VI, describes research on NATURAL DYNAMICS OF BREEDING PARAMETERS IN SHEEP. The study was conducted on 1260 sheep from a zone in North Moldova, in Suceava County, around Ulma and Brodina villages. Monitoring period was

conducted during over a year and started in September 2010. It aimed at oestrus manifestation in ewes, rams mating, assessing the estrus return-rate and surveillance of parturition.

Lactating adult ewes were represented by 59.9%, 20.1% one year ewes and lambs 15.5%. Rams subordinated were 4.5% of the total herd. The main components of the sheep breeds were represented by Țurcana breed and Țigaie breed, 75.8% (955/1260) were Țurcana breed and 24.2% (305/1260) belonged to the Țigaie breed.

Manifestation of spontaneous estrus showed a value of 95.04% for the entire herd. Primiparous sheep showed estrus in 96.9% of cases, 2.5% higher than the average estrous manifestation seen in adult sheep (94.4%). Of the total batch of sheep entered in heat, 3.8% (36/959) developed the heat return syndrome at the end of the mating period. By age, we observed twice the percentage of return rate in multiparous (4.3%) compared to the primiparous sheep (2.01%).

In climate weather conditions during the main breeding season in sheep (9.4°C average temperature, amount of precipitation 2.03 mm/m and cloudiness code 4.6), from N of Moldova, 96.25 % of ewes showed estrus and libido. Total percentage of ewes that gave birth is 86.1%, which represents the monitored batch farrowing rate. Calving rate by age is similar, being 85.9% in multiparous and 86.6% in primiparous. There is a slight positive difference (+07%) for the group of sheep that have calved for the first time. Total prolificacy value was 105%, resulting a total of 868 lambs. We note that 5% of ewes calved twins.

In autumn, Țigaie breed ewes showed heats in 91.7% of cases, compared with Țurcană breed where the percentage was 96.1% sheep in estrus. We notice a positive difference of 5.6% for the Țurcană breed. Țurcană primiparous ewes entered in heat with 6.9% more than the percentage of Țigaie breed ewes (92%). In multiparous, Țurcană ewes came into heat in 95.2% of the herd, with 4.4% more than the Țigaie ewes (91.6%). Țigaie ewes prolificity was 107.8%, 3.8% higher than in Țurcană breed (104%). Number of lambs obtained is also higher, and proportion of twin births was with 5.5% higher.

In Chapter VII, is described ASSESSEMENT OF SEMEN INDICES FROM RAMS DURING PRIMARY AND SECONDARY BREEDING SEASON. Rams were selected for the preparation of monthly spermograms in primary and secondary breeding seasons. The experiment was conducted during the main breeding season in autumn 2010 and during the second mating season in spring 2011.

Within the initiated experiment, were collected a total of 480 ejaculate, 240 from each breed individually, and 120 for each season, breed and ram batch. One ram batch consisted of 5 rams, aged 2, 2.5, 3, 3.5 and 4 years.

In climate weather conditions during the main breeding season in sheep (9.4°C average temperature, amount of precipitation 2.03 mm/m and cloudiness code 4.6), from N of Moldova, following semen average indices were recorded: in Țigaie rams: volume 1.48 ml, 87.69% mobility, concentration 3.36 billion spermatozoons/ml. In Țurcană rams: volume 1.44 ml, 87.28% mobility, concentration 3.57 billion spermatozoons/ml.

In time the weather-climate from northern Moldavia, during the spring season (average temperature 8.5°C, amount of rainfall 0.7 mm/m and Cloudiness code 4.6) following semen average indices were recorded: in Țigaie rams: volume 1.48 ml, 74.00% mobility, concentration 2.05 billion spermatozoons/ml. In Țurcană breed: volume 1.18 ml, 76.17% mobility, concentration of 2.51 billion spermatozoons/ml.

In Chapter VIII, are described QUALITATIVE ASPECTS OF RAM SEMEN WITH EN EYE TO FREEZING.

Researches regarding highlighting the structural changes suffered by ram sperm cells after freezing-thawing, primarily represents a scientific importance for understanding cell ultrastructure in different physiological stages. In 2nd place, the sperm cell ultrastructure knowledge, has great importance in the of freezing-thawing dynamics. Maintaining the structural integrity of the cell, after freezing-thawing, will establish the best methods of freezing, solvent and glycerol levels to maintain cell viability and fecundant capacity.

Sperm cells were frozen in liquid nitrogen, using rapid freezing. Semen frozen in TRIS medium with 4% glycerin showed after thawing a 40.08% motility and cell survival index of 53.03%. Concentration of 5% glycerol in freezing environment is toxic, motility and cell survival after thawing was 10% and 27% lower than average motility and cell survival of sperm cells frozen in glycerol 4%.

Ultrastructural appearance emphasized the following changes in normal cells: plasma membrane is tightly adherent to the head, to the mitochondria and to the axial filament complex. The nucleus shows an intense, electron dense matrix, and acrosome presents an homogeneous acrosome matrix, with electron optic density.

Frozen sperm have normal appearance in 40-50% of them. The rest of the sperm cells have an bloated external plasma membrane, partially or completely detached from the head, intermediate piece and end piece.

In chapter IX, are presented the results regarding REPRODUCTIVE PARAMETERS IN SHEEP, OBTAINED BY OESTRUS SYNCHRONIZATION BIOTECHNOLOGIES. Breeding biotechnologies in sheeps represents the newest and perspective domain of contemporary biology.

STIMULATION OF OESTROUS IN SHEEP DURING SPRING SEASON. Sexual cycle synchronization biotechnology in sheeps, includes oestrus and ovulation synchronization and induction of polioovulation, this is an important stage of artificial insemination biotechnology and embryo transfer, especially in sheeps.

Regimen used was based on Progesterone, prostaglandin F2 α and P.M.S.G. In «urcana ewes, were introduced, intravaginally, sponges impregnated with progesterone for 14 days. In 90% of the experimental group, vaginal discharge observed after removal of inserts with Chronogest, are less abundant and had a more philante character.

In Țurcana ewes from N Moldova, undergoing estrous phase stimulation with Chronogest and PG F2 α , estrus was initiated in 95% of cases, in 5% of them estrus was not present.

BIOTECHNOLOGY OF ARTIFICIAL INSEMINATION IN SHEEPS WITH SYNCHRONIZED ESTRUS. The last of the technological process of artificial insemination is the semen inoculation, very important operation through which semen is deposited in vagina, cervix or uterus.

Ram semen was stored in mini straws of 0.25 ml. When purchasing semen a quality check was performed. Mobility after thawing was between 40 and 45%. Because of the anatomical features of the cervix in ewes, not all A.I. doses managed to be deposited behind the cervix. In these circumstances the A.I. dose was deposited at the cervical opening.

PREGNANCY DIAGNOSIS IN SHEEP BY ULTRASOUND METHOD.

Using ultrasound on small ruminants begins to have great practical importance for uterine health monitoring and gestation diagnosis. Pregnant uterus at 30 - 45 days is more obvious in volume, with thin walls and inside, presence of fluid is observed and on the the echogram that appears in very dark grays tones. Those hypo-echogenic areas (almost anechoic) are fetal fluids.

Embryo at 45 days appears as an hypo-echogenic area, of a few tens of millimeters, surrounded by low echogenic areas. Placentoms in pregnant ewes, appear as lenticular zones of varying sizes (1.5 - 2.5 cm), with significantly increased echogenic areas (wattles), separated by a clear outline and in the central part appear oval areas with low echogenic character (cotyledon).

INDICES OF BREEDING OBTAINED DURING OESTRUS STIMULATION BIO TECHNIQUES AND ARTIFICIAL INSEMINATION.

Fecundity in «urcană batch, assessed on the basis of non return rate (94.8%) is different from the one established dy ultrasonography (89.5%). This difference is not

necessarily false, because the interval between the return to estrus manifestation syndrome and ultrasound diagnosis of pregnancy, a certain period of time passes when the phenomena of fertilization and implantation is possible to be affected. Sheep that reached parturition (calving rate) represented 94.1% and was obtained a prolificity in the experimental batch of 143.7%. Percentage of twin births was 43.75%. All these effects are the result of the hormone therapy to stimulate oestrus and ovulation.

SERUM PROGESTERONE DYNAMICS DURING OESTRUS STIMULATION IN SHEEP.

On day 0 the average progesterone titer was 0.96 ng/ml, immediately after insertion of Chronogest sponges, on day 2, there was a sharp increase in the concentration of progesterone (6.21 ng/ml). Subsequently, it was found a drop in the level of progesterone, but well above baseline (5.08 to 3.22 ng/ml) between days 4 to 14.

From day 15 there a more pronounced decrease in the concentration of progesterone is noticed, concentration which is maintained at a minimum level in the coming days. This decrease in progesterone titer is explained by the removal of exogenous and endogenous sources of progesterone. From the 14th day, with lower progesterone levels, structures of central nervous system are negatively affected, and the cycle resumes by restarting the physiological secretion of Gn-RH. Thus, immediate resumption of sexual cyclicity in the occurrence of oestrus in 36 to 55 hours after regimen completion. Average serum progesterone level on day 16 to 18 was 0.35 to 0.31 ng/ml, a value that explains the effect and results of the regimen applied. During this period the ewes were already in estrus and progesterone levels expresses the value at the first insemination.

This study shows that progesterone introduced by us using Chronogest maintain the progesterone plasmatic concentration over 3 ng/ml after 14 days of treatment, knowing that the minimum level of progesterone after which a response from the hypothalamus can be obtained, is 1 ng/ml.

