SUMMARY

Keywords: Buffaloes, embryo transfer, estrus, reproductive biotechnology.

The doctoral thesis entitled **Research on the implementation of biotechnical methods to stimulate the reproductive function of buffaloes (Indigenous Buffalo Carpatin).**

The paper is structured, according to the rules in force, in two main parts: the first part, entitled "Current state of knowledge" which comprises 46 pages and the second part, "Own research", with a number of 123 pages. The thesis has a total number 200 pages, being illustrated in 116 figures and 33 tables. For its writing, a number of 207 bibliographic title were consulted.

The first part***"Current state of knowledge"*** contains 4 chapters that briefly describe the data related to: the origin and taxonomy of buffaloes, genital morphophysiology in buffalo, parturition and puerperal period and biotechnical methods of directing reproductive function.

The second part "***Own research*** " is structured in 9 chapters organized according to the drafting rules, and includes material and method, results and discussions and partial conclusions. The thesis ends with a chapter of general conclusions, bibliography and annexes.

Chapter 5 is entitled ***The purpose and objectives of the thesis.*** Researcher single countries followed suitability management breeding buffaloes so have applied the tests: -oestrus induction regimens using various methods and choosing the best results; -timing for FTAI with semen; - diagnosis and therapy of ovarian diseases; - the management of the puerperal period ; - diagnosis of pregnancy and its monitoring. The possibility of applying modern reproductive biotechnologies to buffaloes was also pursued . These are : - obtaining and collecting embryos in vivo; - oocyte harvesting and quality assessment; Also, another objective of the thesis was to analyze the macroscopic and microscopic characteristics of the uterus and ovaries. The research was organized in 3 different locations: a farm in Romania, one in Italy, and a research station.

Chapter 6 describes ***Research on seasonal monitoring of buffalo breeding function under natural growth conditions.*** From the point of view of the clinical manifestation of the estrous phase, the characterization of estrus in buffaloes was poorly expressed clinically, reduced vulvar edema, mucus less abundant and reduced in volume. Behaviorally, the buffalo in estrus had less appetite, was slightly agitated and curious. During the seasons, spontaneous estrus was diagnosed at 50.8% in spring, 30.5% in summer, 13.6% in autumn and 5.1% in winter. Frequently the right ovary has a larger size (17.8 mm) than the left one (15.0 mm). During the seasons, spontaneous estrus at BIR was diagnosed at 50.8% in spring, 30.5% in summer, 13.6% in autumn and 5.1% in winter.

Chapters 7 ***Research on the diagnosis, incidence and treatment of gynecological diseases in buffaloes.*** The frequency of placental retention seems to have an increased incidence in the cold months of the years.The differential ultrasound diagnosis between the thecal follicular cyst and the lutein cyst was easy;the lutein follicular cyst has a much thicker wall than the thecal follicular cyst , very clearly visible on ultrasound .Taking into account the seasonality of buffaloes and that the ovaries are small, but with high reactivity, grade I and II hypotrophs can be treated by specific hormone therapy associated with a balanced diet and

neuroreflex therapy. The cases treated with follicular cyst, 40% of the buffaloes are in heat over 21de days, and 17 days 20% of the treated cyst LH (hCG PgF2α).

Chapter 8 details ***Research on the use of biotechnical methods for inducing estrus in buffaloes.*** In order to be maintained a range of 13 to 14 months between calving to buffaloes, insemination has to take place in the range of 85-115 days after calving. The induction of estrus by the administration of luteolytic factor (PGF) to buffaloes has an average value of 58.3% (25% after a single administration 33.3% after the second). Induction of estrus in heifers in low season (summer) has had a successful 88.2%. The gestation rate in the summer season reached values of 76.5% following the directed breeding. In the case of therapy with P4, PGF and PMSG in the summer season, 16.6% of cases of lutein cysts were diagnosed.

Chapter 9 presents **The *challenge of estrus, ovulation and establishing the optimal time for artificial insemination in the Bradano River farm (Italy) (OvS ynch + FTAI).*** The protocol used by us in this study, organizes the breeding activity on the farm, so that there is a balanced correlation between the number of animals, human resources and productivity. Economic efficiency results from maximizing the application of reproductive, biosafety and food biotechnologies. The average fertility in the BR farm in the period 2015-2017 was 73.8%, being diagnosed pregnant with a number of 456 buffaloes from 618 AI. The average fecundity at nullipation registered the value of 78.5%, the primiparous 76.6% and the pluriparous 70.9%. The average total fertility of the warm season was 71.01%, and of the cold season 77.14%. Analyzing the effect of the season on the conception rate, it is observed that in primiparous this influence was considerably higher than in multiparous. In the case of primiparous females, during the autumn period, the conception rate was much higher, with 15% compared to the winter results.

Chapter 10 reveals ***MODAL the increase in the potential of using genetic buffalo use of biotechnology breeding.*** The total conception rate of the Ovsyinch protocol with unicornual FTIA and sexed semen on the Terra di Bufala farm was 45%. By bull categories, the percentages were 40% for Oro and 50% for Aton. At birth, 9 live and viable fetuses were obtained, of which the sex ratio was 88.8% in favor of the female. We state that the goal of increasing the genetic potential of buffaloes by using artificial insemination with sexed material can become achievable.

The development of buffalo embryos evolves about 24 hours faster than bovine embryos. Therefore, the bovine embryo on the 7th day after fertilization is in the stage of expanded blastocyst development, while the same stage is reached in buffaloes on the 6th day of development. The treatment of P4 poliovulation for 11 days, PG-PMSG 2500 IU had a good reactivity obtaining on the day the flushing of four embryos and a birth rate of 25%.

According to the Romanian Embryo Transfer Association (ARET 2019 and AETE Report), the calving of the ***first buffalo calf obtained by ET*** was reported at the Romanian Indigenous ***Buffalo***.

The synchronization of the receptors was done according to the P4-PG-PMSG-HCG scheme, the ovulatory process was 100%, by highlighting the efflorescent CL at 7 days.

The data obtained by us from the embryo-transfer experiments in buffaloes were reported to IETS - the International Embryo Transfer Society of the USA, and published in December 2019, and at the annual conference on January 17, 2020 were published the activities on ET in international level since 2018. In terms of other species - represented by BUFFALO, in Europe in 2018, only Italy (in vitro production) and ***Romania*** (in vivo) reported such procedures.

Due to intermittent follicular waves and with the evolution of a small number of evolutionary follicles doubled and the influence of seasonality, on the surface of the ovaries were identified a small number of antral follicles that could be punctured and aspirated. The method of harvesting by puncture and aspiration of buffalo follicles has a success rate of 45.45%, the identified follicle / oocyte ratio was 11/5. After harvesting oocytes and evaluating their quality, 42.85 % were cultivable oocytes, they have a uniform appearance with similar structure and similar degree of development, representing a population of oocytes that can successfully continue the next stages of in vitro fertilization. Compared to cattle (20-40 oocytes / ovary), the number of oocytes collected is small (9.3 oocytes / ovary), and generates a percentage of less than 50% good quality oocytes that can be cultured and used in the later stages of IVF.

Chapter 11, entitled ***Morpho-functional particulations of the genital tract in the buffalo,*** aimed to identify the obstetrical-anatomical, histological and histopathological features of the ovaries and genital segments.

The morphological peculiarities of the uterus in the buffalo highlight a 7/4 cm cylindrical and cartilaginous cervix, a 2 cm short uterine body with a very strong and 6 cm long intercornal septum, the intercornual ligament accompanies the glued part of the uterine horns on about half of their length, about 8 cm, uterine horns of 10 cm have a crunchy and sinuous tubular appearance with many bumps. The symmetry of the uterine horns at 2 months of gestation is still maintained, with a diameter of 3 cm, the uterine wall of 3 mm is firm and tonic, the embryonic sac with the same size as the uterus after sectioning is highlighted and the embryo has a size of 2/1 cm . On average, the buffalo ovaries recorded the following morphometric landmarks: length - 24.3 mm, width - 18.25 mm, thickness - 13.15 mm and a weight of 4.53 g. The diagnosed ovarian diseases were grade ovarian hypotrophy. I (14.51%), cyst Ovari is ni (11.29% from the 42.86% of the corpus luteum cyst, 28.57% and 28.57% lutein cyst follicular cyst) and Ovaro-bursal adhesions (1 61%).

Chapter 12 describes the ***Use of ultrasonography in monitoring buffalo reproductive function.*** Results of gestational diagnosis and identification of fetal sex (ultrasonographic sexing) are presented.

Due to the tonicity of the uterine body and the thicker uterine wall, the uterine asymmetry between the two horns appears noticeable later than in cows, in the interval of 60-70 days. The accuracy of the diagnosis of gestation in the Romanian native buffalo (BIR) by transrectal palpation in the period 50-60 days after estrus was 70.83%, and in the period 60-70 days it is 98.04%. The accumulation of fetal fluids is low until day 40 of gestation, but subsequently there is a significant increase in fetal fluid volume and fetal size, with a consistent increase in uterine size.

Both male and female genital tubercles appear on the screen as bilobed structures whose echogenicity is similar to that of bone tissue. Examination of buffaloes for fetal sex can be done between days 60 and 120 of gestation, but the ideal window of opportunity is between days 70 and 85. Even if the genital tubercle is visible from day 51, it does not reach its final position before day 58.