

Red deer (*Cervus elaphus L.*) live in swarms during winter and spring. At the turn of July and August the swarms disintegrate. Males of deer start the migrations – around September/October. After rut, the hinds remain in swarms. Males of red deer begin reproductive activity at the age five years old, and hind puberty is reached in the age of 16th month. The length of the estrous cycle is from 18 to 22 days. The pregnancy in hind continues for 231-238 days. Delivery occurs in May or June.

Hunting is an integral branch of forestry, and wild animals constitute an important part of forest ecosystem. The wild animal management is regulated by hunting laws and adequate breeding plans, which take into consideration both protection of the wild animals and control of its populations as well. Based on the annual calculations of Polish Hunting Association, in Poland 200,000 of red deer were noticed in 2016/7 season. In area of Warmia and Mazury in season 2016/17 about 22 thousands red deers were noticed, with acquisition of animals on the level 6,860. There is necessity of development of sustainable reproductive regulation, preceded by knowledge of the reproductive processes of the red deer. In Poland, deer farms are established and New Zealand is a giant in this branch of animal production, with a population of more than 2 million deer, while in Poland it is estimated at 5,000 animals (data from 2015). The history of breeding in Poland is about 50 years, and the relatively short period of breeding compared to breeding domestic animals causes the breeder to meet many challenges. In addition to information on the duration of pregnancy and reproductive seasonality, there are no data on the mechanisms and factors involved in regulating the reproductive processes of these ruminants. It is not known what factors in the reproductive system are involved in regulating the cycle (estrus and anestrus phases), implantation, embryonic development of the embryo, the biological characteristics of ovarian and spermatozoa have not been recognized for their usefulness for fertilization. There are no markers for reproductive status (no pregnancy / pregnancy) female deer. The specificity of studies conducted on slow-growing animals is limited to access to the test material and it is therefore necessary to introduce methods of reproductive parameters in a non-invasive way (feces).

Scientific aims:

- Determination of hormones and cellular mechanism involved in regulation of reproductive processes in non-pregnant and pregnant hinds,
- Evaluation of methods of receiving gametes and its characteristic.

Project's research hypotheses:

- 1) The results allow determination of endocrine regulation of hormone production and expression in the reproductive tract in hinds, such as: hormones of hypothalamus - pituitary – ovaries axis, reproductive seasonality and cyclicity, implantation period and pregnancy development.
- 2) Characteristics of oocytes allow for recognition its quality and the level of maturation.
- 3) The results of the study will support the determination of fertilizing capacity of stored epididymal sperm during *in vitro* and *in vivo* insemination.

Experimental material will be collected *post mortem* right after shout during hunting from wild animals (hunting season in Poland for hinds: 1st September – 15th January) and it will be: peripheral blood, reproductive tissue fragments (uterus, placenta, ovary) and feces from hinds. Moreover there is planned collection *post mortem* of reproductive tract tissues, semen and blood from farm animals (females and males of red deer).

Analytical methods: concentration of hormones (EIA/RIA), mRNA expression (Real Time PCR) and protein expression (Western blotting) for steroids, interferon tau, prolactin, and eicosanoids (prostaglandins, leukotrienes), FSH, LH and parameters of oocyte and blastocyst quality, immunohistochemistry in uterine tissue. Sperm motility parameters (CASA system), sperm plasma membranes integrity, the activity of mitochondria microscopic observation), the activity of antioxidant enzymes, lipid peroxidation in sperm homogenates (spectrophotometrically).

Proposed studies will contribute to define molecular mechanisms that regulate reproductive functions in ruminants in Poland. Studies on the mechanisms of reproductive regulation and gamete characteristics, attempts at *in vivo* and *in vitro* fertilization of red deer can be a research model for endangered Cervidae research in the world.