The course of the fertilization process and the molecular mechanisms that guide it in birds is not fully understood. In the natural environment, birds only reproduce under favorable conditions. In breeding, the reproduction of these animals is programmed and controlled. Proper breeding of birds is important because it affects the production, health, and profitability of the flock. The key element of the reproduction process is the fertilization. This phenomenon consists of the combination of cytoplasm (plasmogamy) and cell nuclei (karyogamy) of mature male and female reproductive cells. It initiates the creation of a new organism (5, 13). Precise determination of the mechanisms leading to fertilization in birds is important in the programming and control of breeding in farms, but also in the creation of transgenic animals and the reproduction of species threatened with extinction.

The encounter of mature male and female reproductive cells in birds

The precondition for the fertilization to take place is the encounter of mature reproductive cells. The gametes attain their maturity partly during development. In male birds, sperm is produced in paired testes located in the abdominal cavity (Fig. 1A). Then they are shifted to the paired epididymis and the long deferent ducts. Their endings expand to form seminal vesicles in which sperm is stored. In the deferent duct, male gametes are formed in paired testes located in the abdominal cavity. Sperm cells in the female reproductive tract do not require capacitation and are already fully capable of fertilization. As a result of internal insemination, male reproductive cells enter the oviduct. In this organ, they are selected and stored in the primary and secondary sperm storage tubules of the mucous membrane. They are released in batches shortly before ovulation. After reaching the oocyte, the sperm binds to the IPVL. This induces an acrosomal reaction that allows the male reproductive cells to penetrate to the surface of the oocyte, especially at the germinal pole. Next, as a result of physiological polyspermy, many sperm cells reach the ooplasm where they form haploid male pronucleus. This phenomenon is necessary to activate a polylecithal egg and produce a haploid female pronucleus. In the final stage, the female pronucleus merges with the single male pronucleus, which leads to the formation of a diploid zygote. The excess male pronuclei present in ooplasm are broken down by endonucleases (DNases). Understanding the mechanisms leading to the interaction between sperm and oocyte in birds may allow for more accurate programming and breeding of these animals in poultry farms and the introduction of extracorporeal fertilization techniques. In addition, it could be useful for the reproduction of endangered bird species.

Keywords: fertilization, bird, physiological polyspermy
In females of most species of birds, gametes usually develop in the ovarian follicles of the left ovary. Oocytes are polylecithal, and yellow yolk accumulates in the form of a large ball on the vegetal pole. The animal pole concentrates the cytoplasm with the cell nucleus in the form of a germinal disc (5, 15). The oolema surrounding the yolk is discontinuous and produces single microvilli. Above the germinal disc, the cell membrane is continuous and forms numerous, branched microvilli directed towards the perivitelline space. This space can be seen between the oolema and the inner perivitelline layer (IPVL), which is formed in the ovary around the oocyte as the primary egg shield (Fig. 3) (3, 5, 17, 18, 28). Under the influence of hormones the mature ovarian follicle ruptures and releases the secondary oocyte in a metaphase of the second meiotic division (ovulation). The ovum, surrounded by the IPVL, enters the infundibulum of the left oviduct. Its here fertilization occurs. Afterward, the fertilized oocyte goes through the following sections of the oviduct, i.e. magnum, isthmus, uterus, and vagina joining the cloaca (Fig. 2) (2, 5). During migration, the ovum is surrounded by secondary egg shields. Their task is to protect the embryo and allow it to grow outside the mother’s body at the expense of material collected in the form of yolk (5, 7, 15).

**The migration of sperm in the female reproductive tracts of birds**

In birds, the encounter of mature male and female gametes is made possible through internal insemination. Sperm cells are introduced into the female’s cloaca during copulation. This process is not synchronized with ovulation (2). Male gametes reach the vaginal part of the oviduct near the uterus. This place is described as the utero-vaginal junction (UVJ). This section of the oviduct is approximately 2-3 cm long (20, 28). The epithelium of mucosa of UVJ forms crypts with a length of approx. 200 µm called primary sperm storage tubules (SSTs) (2, 20, 28). Immediately after copulation, sperm cells gather in large numbers in SSTs creating the so-called seminal nests (5, 6). There is a suitable microenvironment inside the tubules, thanks to which the male gametes retain their lifespan from 2-3 weeks (rooster) to 15 weeks, e.g. turkey (2, 6, 20). Sperm cells from seminal nests are released in batches before each ovulation (9, 20). Even before the ovarian follicle ruptures, motile reproductive cells migrate to the infundibulum, where they penetrate into the invaginations of the mucosal epithelium, the so-called secondary SSTs. These structures are short and accumulate only a few sperm cells (28).
Sperm penetration of the IPVL of avian oocyte

During fertilization in birds, a multistep interaction of the sperm with the oocyte takes place. In the first phase, male gametes penetrate through the IPVL. This process precedes sperm binding and the induction of an acrosomal reaction in it. The IPVL is fibrous. The fibers are made of glycoproteins, mainly ZP1 and ZP3. In addition, ZP2, ZP4 and ZPD are present in small amounts (11, 14, 17, 18). The ZP1 and ZP3 glycoproteins are interconnected and in this form they get incorporated evenly into the IPVL around the ovum (17, 18). These compounds function as receptors and binding sites for sperm (4, 10, 28). However, ZP2 gets located only in the area of the germinal disc and can act as a factor attracting male gametes close to the germinal disc of oocyte (11, 16). The binding of sperm cells initiates a cascade of reactions in them leading to the release of enzymes from the acrosome and the unveiling of the inner acrosomal membrane. In birds, this process does not require sperm capacitation (21, 28). The N-glycans of the IPVL glycoproteins, mainly ZP1, induce an acrosomal reaction (7, 11, 14, 28). The release of proteolytic enzymes from the acrosome and the unveiling of the inner acrosomal membrane most likely occurs after the top part of the head is detached as a result of membrane fusion in the rear region of the acrosome (1). The acrosomal reaction allows sperm penetration through the IPVL. Male gametes pave the way by engaging the participation of a multi-enzymatic protease complex. These enzymes break down ZP1 into single amino acids and peptides and lead to the release of ZP3 (11). The sperm hydrolyse a hole of approx. 9 µm diameter and within about 3 minutes it passes through the IPVL (25, 28). Sperm cells have a limited time of interaction with the IPVL. Within 15-30 min. after ovulation an outer perivitelline layer (OPVL) around the oocytes is formed (8, 25, 27, 28). The OPVL acts as a mechanical barrier for sperm and the enzymes contained therein inhibit the induction of the acrosomal reaction (Fig. 3) (23, 25, 27).

Penetration of multiple sperm cells into the ooplasm of avian ovum

Male gametes, passing through the IPVL at the germinal pole, enter the perivitelline space. In birds, the phenomenon of physiological polyspermy is observed. Multiple sperm cells interact with the oolema covered with numerous microvilli and penetrate into the ooplasm (9, 12, 24, 28). Sperm penetration occurs through the fusion of the inner acrosomal membrane with the oolema and by phagocytosis. Whole male gametes are absorbed into the ooplasm, which leads to plasmogamy (Fig. 3) (13, 28). Polyspermia facilitates fertilization of polylecithal ovum, due to the short duration of the interaction of male gametes with the IPVL (9). Only one of these gametes participates in karyogamy. The remaining numerous male gametes are necessary for the activation of the oocyte. Their presence contributes to the increase in the level of calcium ions in the ooplasm. This triggers enzymatic reactions that allow the completion of the second meiotic division by the secondary oocyte (5, 9). In addition, sperm contains C zeta phospholipase, aconitase, cytidine synthetase, which are

Fig. 3. Oocyte interactions with sperm in birds: 1 – the surface of the oocyte surrounded by IPVL, 2 – sperm binding to IPVL, 3 – acrosomal reaction and sperm penetration of IPVL, 4 – sperm interaction with the oolema, 5 – penetration of sperm cell into the ooplasm
Explanations: IPVL – inner perivitelline layer; OPVL – outer perivitelline layer
also included in the activation factors (7, 9, 11). In chickens and turkeys, at least 6 sperm cells have been shown to be necessary for the proper course of the fertilization process (26, 28).

**Formation of pronuclei and karyogamy in birds**

The sperm cell nuclei present in ooplasm undergo numerous structural changes leading to the formation of haploid male pronuclei. A haploid ootidote develops as a result of oocyte activation after the sperm transformation. Also a nuclear envelope is formed around the female nuclear material, resulting in the formation of the haploid female pronucleus at the center of germinal disc (5, 28). Male and female pronuclei combine in the process of karyogamy to form the zygote diploid nucleus. At the same time, a spindle is formed from the centriole brought by the sperm, which initiates numerous mitotic divisions of the germinal disc and the creation of a new complex organism (22, 28). One male pronucleus, which most probably arises from the sperm bound to the oolema located directly above the nucleus of the oocyte, is involved in karyogamy. The genetic material of the extra spermatozoa is degraded by DNases I and II present in the ooplasm at the early stage of embryonic development. This phenomenon inhibits pathologial polyspermy (15, 23, 24, 28).

Avian sperm cells are homogametic (ZZ), while the ova are heterogametic (ZW). The male gamete carries Z-heterochromosome, whereas the ootidote carries the Z or W heterochromosome. The presence of ZZ chromosomes leads to the development of paired male gonads-testes, while ZW leads to fully developed left ovary and oviduct (5).

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