

Effect of Consuming Maize Infested With *Fusarium* Mycetes Upon The Follicular Pool in Gilts

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Abstract. Maize utilized in combined fodder fed to 80 young sow aged 4 months meant for reproduction and displaying clinic signs of hyperestrogenism, was checked for mycological content. The examination has revealed a large load with mycetes of genus *Fusarium* (200-5400 thousand UFC/g). The clinical examination showed genital infantilism, tumefaction in the vulva, an exaggerate development of the mammary chain with conspicuous mamillary tumefaction. The histopathological examination displayed an extremely intense ovarian activity with numerous follicles in all stages of development but no yellow body, demonstrating that no follicle has reached ovulation. They finalize through follicular atresia a phenomenon which could engulf follicles in any stage of development. The absence of ovulation is due to intense-, persistent estrogenic effect induced by mycotoxins produced by genus *Fusarium* (zearalenone) resulting in running out from the follicular reserve within very short space of time in the very young animals, thus practically compromising them from reproduction point of view.

Key words: *Fusarium*, zearalenone, follicle atresia, ovary, apoptosis

INTRODUCTION

Follicular atresia is a physiological process taking place in the ovaries of mammals with the scope of gradual consumption of the surplus of ovarian follicles being normally possessed by any female. Of the several hundreds of thousands of primordial follicles extant the ovaries of mammalian animals, only a very few numbers (0.5% in bovine) evolve to reach the phase of dehiscent follicle, the others being ousted through follicular atresia (Irving-Rodgers, 2001). Follicles can be engulfed by atresia in any stage of development (Knobil, 1994), and the process goes on normally from puberty up to the depletion of all follicles in the ovaries (menopause). Beside physiological follicular atresia, there are situations when certain pathological factors may trigger and sustain processes of atresia. Of these, a special place is being detained by mycotoxins, mainly zearalenone (ZON) produced by *Fusarium* mycetes (Gaumy et al., 2001). Ingestion of fodders contaminated by such mycetes (mostly *Fusarium graminearum*) causes disturbances in the function of reproduction with a large number of species. The primary effect of ZON is estrogenic and the mostly affected farm animals are the sexually immature gilts. (Gaumy et al., 2001) This estrogenic effect is due to a similarity in structure between ZON (and its metabolites) and estradiol (Osweiler, 2000). α -zearalenone is estrogenically three times more active than ZON, and the relative affinity to connect to estrogenic receptors is higher in pigs than the other species (Reishauer, 2005). ZON is rapidly absorbed and ousted. It is metabolized in the liver (possibly intestinal mucosa) and eliminated as glucuronic compounds through urine and faeces after enterohepatic recycling (Diekman, 1990).

MATERIALS AND METHODS

The biological material utilized in this study was represented by 80 young sows aged 4 months from a farm in County Cluj; the animals displayed clinical signs of hyperestrogenism. The combined fodders fed were mainly made up of maize. The mycological examinations of maize samples were carried out by inseminating them on sterile Petri dishes, 10 cm in diameter, two per each dilution, by introducing 1 ml suspension from the dilutions of 10^{-4} , respectively 10^{-5} . The culture medium utilized was Sabouraud. The mycological examination had that the maize samples were infested by *Fusarium* mycetes, known as phytoestrogen agents. Identification of fungi was implemented *via* determination of the macroscopic culture traits and examination under stereomicroscope. To carry out histopathological exams, there were drawn the ovaries of 4 young sows by means of lateral abdominal laparoscopic ovariectomy, set in 48- Stivie mixture and embedded in paraffin. 5 μ -series sections stained with Goldner's trichrome method were utilized.. The effect of toxin on the structure and functioning of ovaries in the animals in a special hormonal situation was analyzed on histologic sections.

RESULTS AND DISCUSSIONS

The maize samples analyzed displayed a heavy mycotic load, the UFC/g varied between 200 thousand and 5,400 thousand. The examination of culture traits by means of magnifying glass with stereo lights of preparations on blade-cover glass (extracted from culture), have revealed that mycetes of the largest occurrence was of genus *Fusarium*.

Clinically, one can notice genital infantilism, tumefaction of vulva, an exaggerated development in the mammary chain, mamilla swelling, as well as the presence of mammary nodules of several size (rice/millet kernel), nodules and mammary cysts. .

The histological exam of ovaries revealed a generalized heavy congestion, of even congestive-hemorrhagic aspect, in certain areas. Ovarian activity shows much more intensely than normally as there exist countless ovarian follicles in varying phases of evolution or involution, of majority being the cavitory follicles (Fig.1.)

Primordial follicles are surprisingly few for so young animals, but similarly with the existing ones the majority presents alterations, either cytoplasmatic ones, shown mainly by vacuolar degeneration (Fig.2.), or nuclear, represented by vacuolization, karyopyknosis or karyorrhexis (Fig.3.)

With primary follicles the most obvious alterations show up with ovocytes and present a stage evolution. Initially, a condensation is seen in the nuclear chromatin, then a fragmentation of it (karyorrhexis). Concurrently, the cytoplasm gradually vacuolises (Fig.4.). Then, the nucleus becomes disorganized (karyolysis) and the cytoplasmic vacuolization advance so that, at a given moment, the cytoplasm reveals a reticular aspect. The follicular cells do not show signs of degeneration discernible by optical microscopy. With the cases studied by us, essential follicles were not of majority. It seems to be linked to the doses of zearalenone, as shown by Wasowicz et al., (2005) who, by having utilized various doses of ZON found that with a doses of 20 μ g/kg one cannot find exaggerated ovarian activity ; however, with 40 μ g/kg numerous pre- antral follicles, both evolving and atretic, show up. Secondary follicles show the nuclear- and cytoplasmic modifications described with the primary ones, however, of higher intensity. With the cases in study, the antral follicles were the most numerous, suggesting that the ZON doses was higher than that engaged by Wasowicz et al. With this category of follicles there are to be discovered alterations in the

follicular cells in the granulosa-theca and their intensity being very different from one follicle to another being more advanced, as the follicle is in more advanced phase of evolution, and the evolution is stage. In the initial stage, there is to be found an exaggerated proliferation of cells in the granular sheath which, at a moment, reaches a constitution of 12-15, and sometimes even of 30 rows of cell, as to 3-4 normally extant (Fig.5.). Moreover, even with this stage, cell proliferation is in full development, fact illustrated by the existence of numerous cells in mitosis. In a somehow more advanced stage, the granular cells are gradually caught in a degenerative process. Initially, the surface cells are affected (from the follicular cavity) which undergo nuclear and cytoplasmic alterations, and lose the connection with the other cells and turn into apoptotic bodies noticeable either in the superficial area of the granulosa or free in the follicular cavity (Fig.6.). The process of apoptosis grows visibly in intensity and extends gradually to the cells in the central area, even in the basal one, of the granulosa. The phenomenon gains momentum and shortly all the cells of the granulosa will be caught in apoptosis and this membrane disappear as entity (fig.7.). Concomitantly, the Slavjansky membrane will also disorganize, so that the follicular cavity hung with tapestry, in this stage by the inner conjunctive theca. Simultaneously, on the level of the inner conjunctive theca, an intense proliferation of fibroblasts is taking place. These will gradually invade the follicular cavity and, by intense collagen synthesis, successively fill the cavity with conjunctive tissue. This has -at the beginning- a loose countenance which progressively consolidates and organizes in thick, dense fibers, so that, in the end, the former follicle gets fibrous (Fig.8.). Of all follicular components, the membrana pellucida is the most degradation hardly noticeable (collapsed and thickened) even in the stringy follicles.

The aspects noticed, due to histologic exams, confirm the state of hyperestrogenism found also with the clinic exam. Pronounced and persistent hyperestrogenism induces and exaggerated, abnormal ovarian activity. In the special situation created, the essential follicles enter successively and galloping the follicular evolution processes, covering one or more phases but no one reaches the stage of dehiscent follicle. Their evolution ceases in a certain moment, differently from one follicle to another followed by the transformation into atretic follicle. The unreeling of the processes of atresia differs with the stage the follicle got to at the time of initiation of atresia. They begin, with all follicles, in structural alterations in the oocyte. Follicular cells show only discreet alterations with the small- or medium size ones, as the process is similar in many concerns with the common follicular atresia (Pastor, 1999). The net difference is given by the very large numbers of atretic follicles, both pre-antral and antral. With cavitory follicles, the degenerative processes in the oocyte are rather rapidly followed by structural alterations in the cells of the membrana granulosa. Although starting later, the degenerative processes in the granulosa cells unfold very rapidly so that this membrane vanishes as entity more rapidly than the degenerate oocyte. Unusual also is the manner of finalizing the follicular atresia, owing to the effect of zearalenone, with intense fibrosis of the zone and not the reorganization of the ovarian stroma, as it happens with the physiological atresia.

The features encountered suggest the existence of an exaggerated and persistent estrogenic stimulus which initiates, maintains and accelerates the processes of follicular evolution. The persistency of a high estrogenic level continuously drive other follicles which develop to a certain stage, being in the end eliminated by apoptosis. Concomitantly, the keeping high of the estrogenic level decides that no ovarian follicle reach the final, ovulating stage. The total absence of ovulation is illustrated by the fact that neither the functional- nor the involuting yellow bodies were revealed.

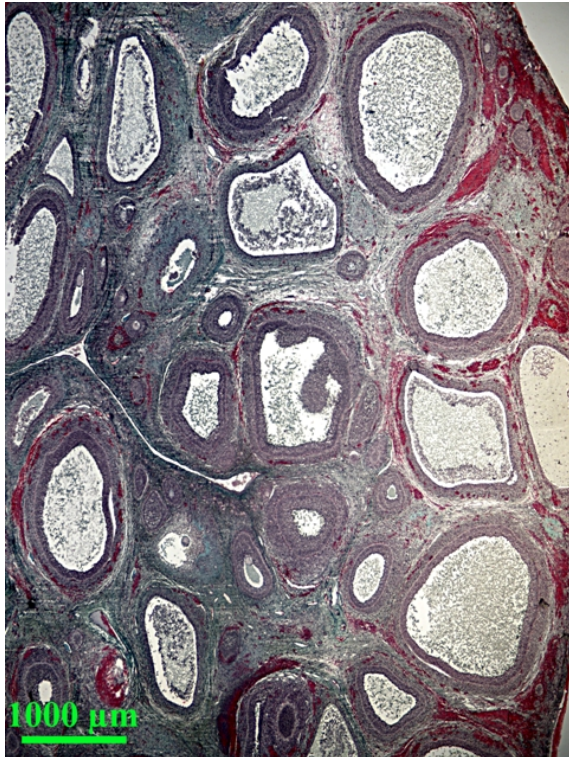


Fig.1. Tricrome Goldner (ob, 2X). Ovarian follicles in different stages of evolution

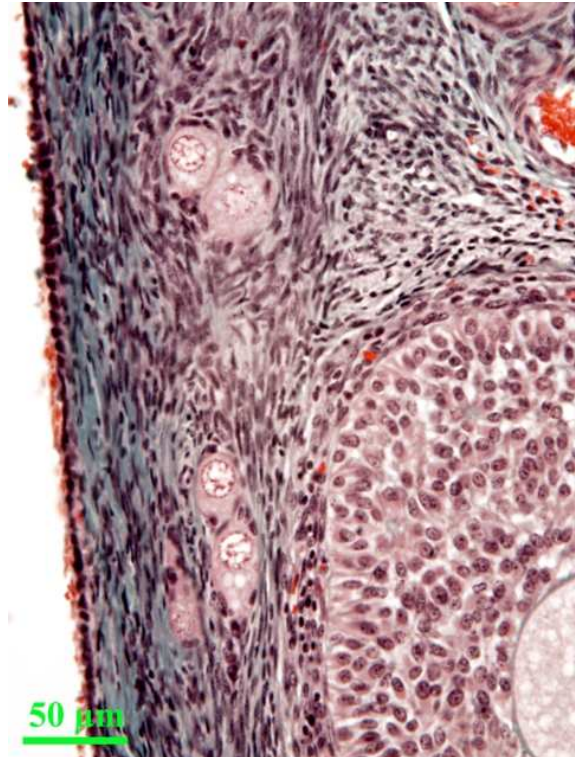


Fig.2. Tricrome Goldner (ob, 40X)
Vacuolar cytoplasmic degeneration
in primordial follicles

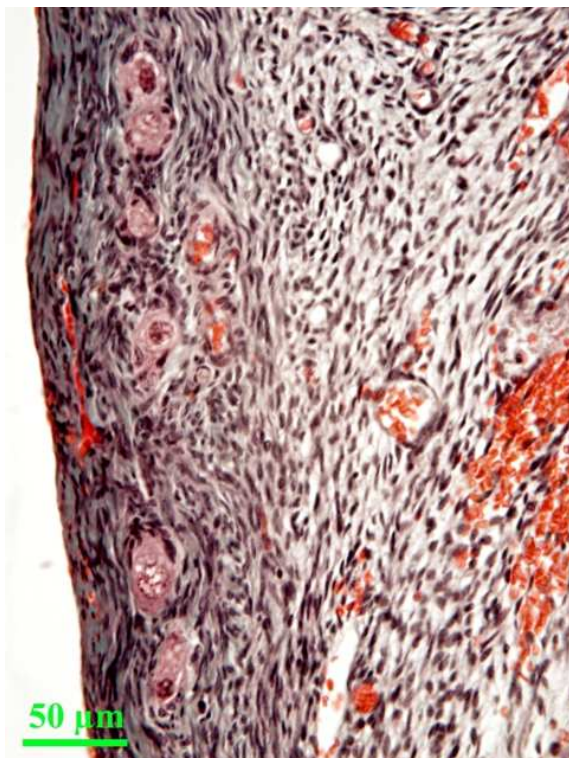


Fig.3. Tricrome Goldner (ob, 40X).
Karyopyknosis and Karyorrhexis in
primordial follicles

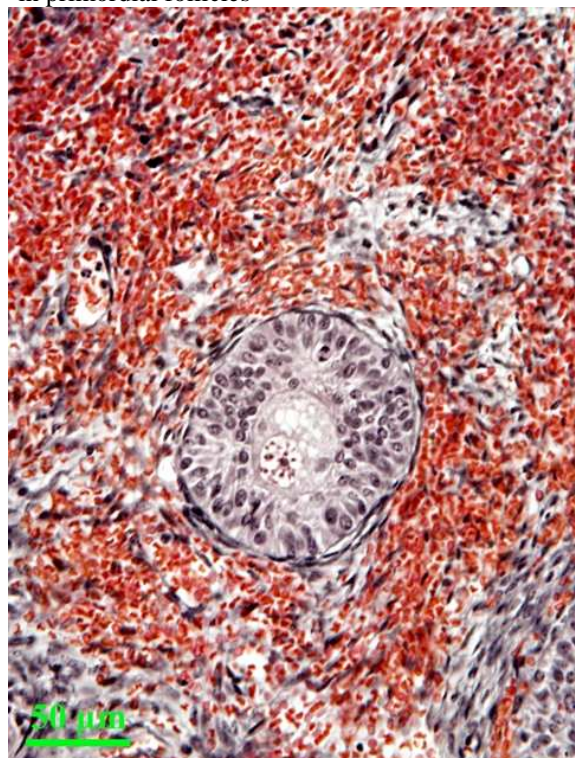


Fig.4. Tricrome Goldner (ob, 40X). Vacuolar
cytoplasmic degeneration and karyorrhexis
in primary follicle

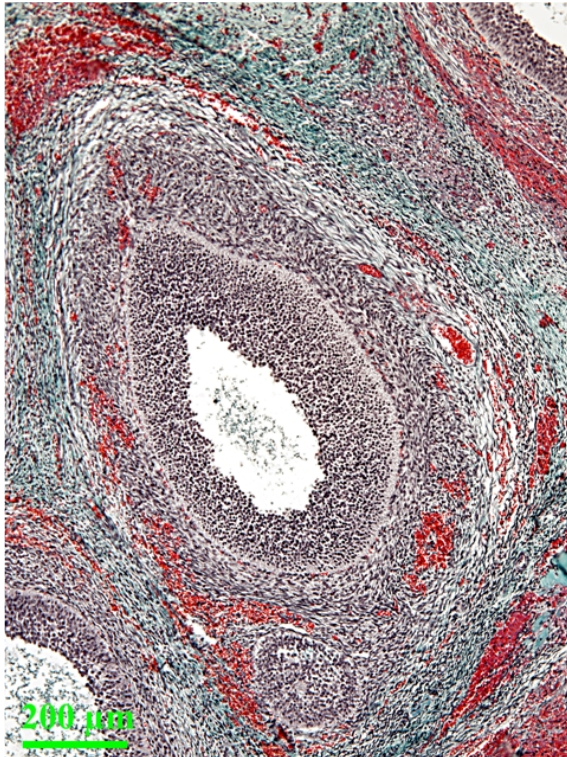


Fig.5. Tricrome Goldner (ob,10X). Intense proliferation of cells in the granulosa-theca

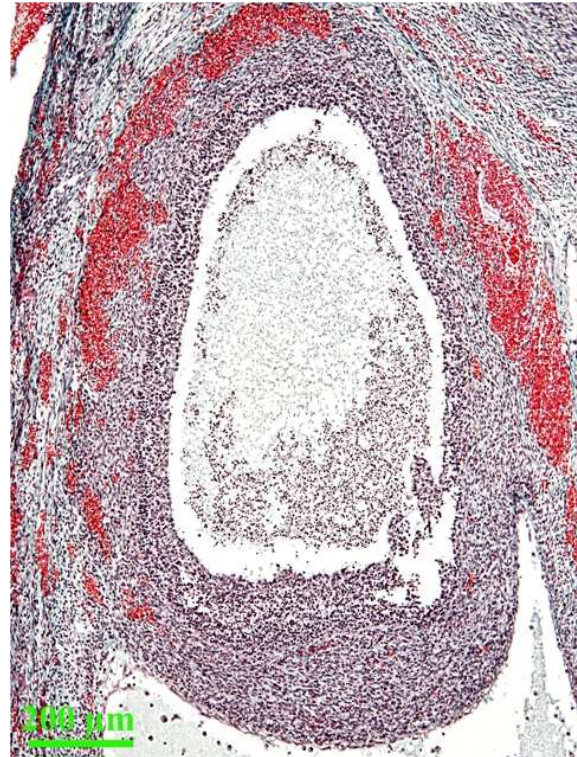


Fig.6. Tricrome Goldner (ob,10X). Massive apoptosis of cells in the granulosa-theca

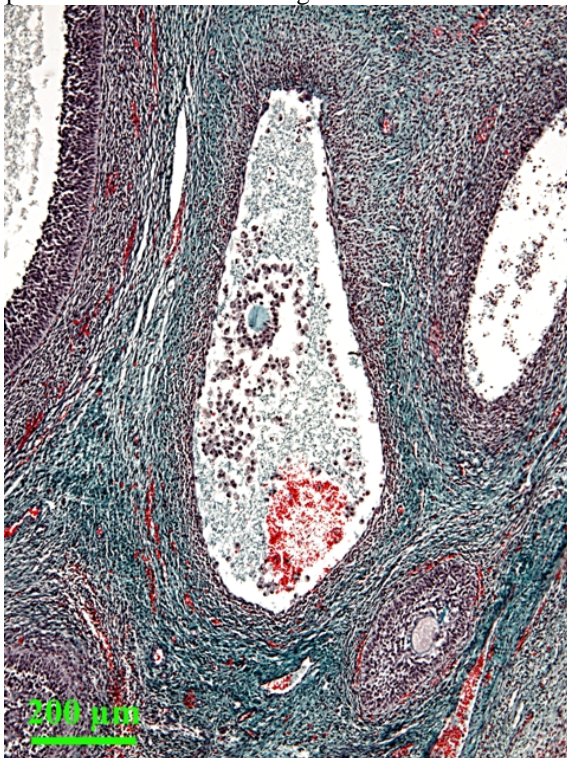


Fig.7. Tricrome Goldner (ob, 10X). Hemorrhage in follicular cavity and granulosa-theca disappearance

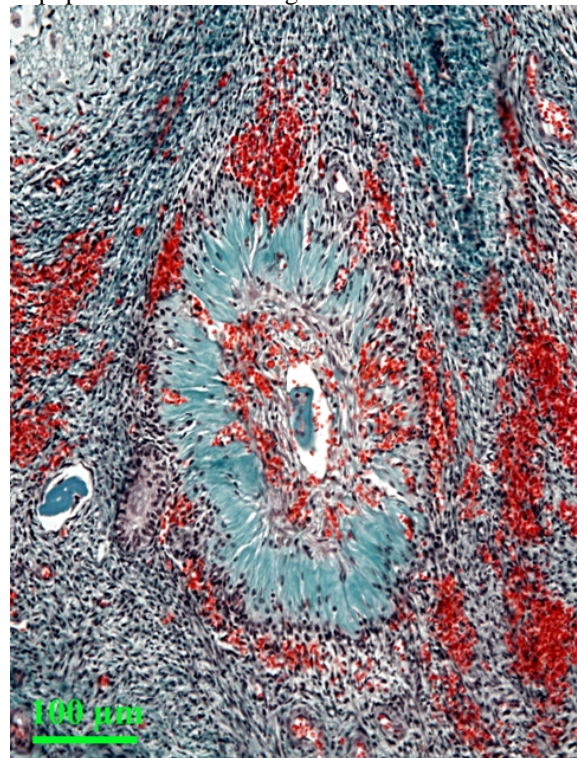


Fig.8. Tricrome Goldner (ob, 2X). Follicle fibrosis

The rapid depletion through atresia of the ovarian follicles will cause the follicular reserve in the ovaries to drop alarmingly, within a very short space of time. The drastic

reduction in the follicular reserve will end in compromising the females from the point of view of reproduction, at an early age.

CONCLUSIONS

The results of mycological examination of the maize samples under study have revealed a very high mycotic load (200-5400 thousand UFC/g) with the prevalence of genus *Fusarium* mycetes, in spite that the maize did not present organoleptic alterations macroscopically detectable.

The histological examination of ovaries acknowledged the condition of hyperestrogenism noticed on clinical- and paraclinical examination, the follicles alertly entering processes of follicular evolution but none managed to finalize through ovulation.

The ovarian follicles undergo processes of atresia of stage evolution and are characteristic in accordance with the stage the follicle is at the time of initiation of atresia.

The pathological atresia defined by the action of the zearalenone is finalized through intense fibrosis of the area taken by the respective follicle and not through reorganizing ovarian stroma, as it goes with the physiological atresia.

The state of persisting hyperestrogenism establishes the severe drop in the follicular reserve within a very short space of time as well as compromising the number of young sow for reproduction. .

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