A CASE OF FAILURE OF ARTIFICIAL INSEMINATION CAUSED BY SUBFERTILITY OF MALES DUE TO IRREVERSIBLY INJURED TESTES

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Abstract - A clinical case of subfertility of males is described. It occurred in a commercial rabbitry of 1200 females and 72 males. After the first two inseminations where the quality of undiluted semen was evaluated as 5 scores of PetitJean scale, the score dropped suddenly down to 1.5-3. Ten males were culled. The samples from testes and internal organs were taken and processed by classical histological techniques. In all examined testes thickening and hyalization of basement membranes of seminiferous tubuli were observed. In germinal epithelium of almost all tubuli crosses a few spermatides and very numerous primary spermatocytes forming a thick layer were present. The lumen of tubuli was filled with cell debris as well as cell shadows. Decrease in the intertubular tissue and number of interstitial (Leydig's) cells was found. This histological picture indicates that spermatogenesis slowed down. This process takes place under the control of FSH, so such a picture should be seen when FSH deficiency appears. FSH deficiency can be provoked by exogenous estrogens. Zearalenon (F-2 toxin) is known for its strong estrogenic activity. The animals were fed with mouldy feed that could have contained F-2 toxin so the hypothesis about zearalenon intoxication as an agent of irreversible changes in testes is presented.

INTRODUCTION

The number of industrial rabbitries using artificial insemination technique has significantly increased for some years. Artificial insemination is considered in rabbit breeding - like in other animal production - to be very useful and there is no need any more to convince the breeders to introduce it. Nevertheless there are some conditions necessary to make this technique easy and efficient. The first one is to obtain semen of the best quality. When the reproduction disorders appear the breeders usually look for the reason in females, in their sterility or subfertility. This is probably why the literature contains no references to clinical cases of bucks subfertility. Another reason for this fact is that it is very difficult or impossible to diagnose subfertility of bucks being used for natural covering, because high concentration of spermatozoons hides their low quality.
Therefore it was found useful to record the clinical case where the drop of breeding productivity was caused by subfertility of males due to pathological changes in testes.

DESCRIPTION OF THE CASE

The case occurred in a commercial rabbitry of 1200 females and 72 males, 3 months after the farm had started working. The animals of a commercial rabbit strain were put into the rabbitry in question in the beginning of December. The females were 8-10 weeks old and the males 12-14 weeks old. They were fed with commercial pelleted, balanced feed *ad libitum*. The mean weight gains were correct, the animals grew up in time and the first insemination took place at the age of 16 weeks for some females, and 18 for the others, when they weighed at least 3,2 kg.
Mortality throughout this period was normal.
About 3 weeks before the first insemination (mid-January) there was ventilation break-down in the males' room followed by respiratory infection in 18 males. In laboratory examination *Pasteurella multocida* was confirmed as the agent of the infection. The sick animals were treated with oxytetracyclines (Engemycine Intervet).
The males were prepared for the first insemination by collecting the semen twice a week during the 2 weeks proceeding insemination. The females were divided into 2 reproduction groups, each one managed in single schedule reproduction system. The males were sampled twice a week between 2 successive inseminations. The quality of undiluted semen was evaluated using the PetitJean scale. The mean score of the semen obtained between the beginning of February and beginning of March was 5.1. Heterospermie was used. The results of the first insemination were 65% of gestation and of the second one - 85%.

CLINICAL SYMPTOMS

Two weeks before the first symptoms appeared a new supply of feed had been delivered. The feed was wet, mouldy, smelling badly and pellets were soft, disintegrating when touched. While waiting for a new supply of feed the animals were fed with the old feed mixed with mouldy pellets. This lasted for 5 days. 24 hours after giving the mouldy feed the animals presented inapetence and depressed demeanour. After a few days the loss of weight was apparent, but they showed no evidence of fever or any other constitutional symptoms. The rabbits were normal in all other respects. They recovered 10-12 days after the spoiled feed had been changed.
The semen obtained on the 2nd, 6th, 8th and 10th of March gave the following scores : 4.8, 6.2, 5.5 and 5.9 respectively, that means better than the scores from the entire period in question. On the 11th of March this score dropped down to 3 and remained at this level on the 15th and 17th of March. On the 18th of March the quality of undiluted semen dropped down again to 1.5. On the 2nd of April a slight improvement was observed (3.5). Afterwards the score fell again to 1.5-1.8 and this level was maintained during the next month. Numerous spermatozoons with cytoplasmatic drop were found in all semen samples. It was decided to cull 10 males chosen at random.

MATERIALS AND METHODS

The samples from testes, small intestine, liver, kidneys and lungs were taken. The testes were fixed in Bouin's fluid. The other samples were fixed in 10% formaldehyde (pH 7.4). All samples were processed by classical histological techniques : embedded with paraffin method and stained with HE and PAS by McMANUS.

RESULTS

Post-mortem examination showed foci of bronchopneumonia in the lungs of 8 animals. In other organs no lesions were found. Microscopic examination of lungs resulted in "*peribronchitis nodosa* et *bronchitis desquamativa*" and "*pneumonia catharrofibrinosa*". Sections of livers from 2 animals showed foci of hydropic degeneration. The intestines of 3 animals were slightly oedematous and desquamation of epithelial cells was observed. No lesions were found in kidneys. In all examined testes thickening and hyalization of basement membranes of seminiferous tubuli were observed. In germinal epithelium of almost all tubuli crosses a few spermatides and very numerous primary spermatocytes forming a thick layer were present. The lumen of tubuli was filled with cell debris as well as cell shadows. Decrease in the intertubular tissue and number of interstitial (Leydig's) cells was found.

INTERPRETATION

The low number of spermatides indicate that spermatogenesis slowed down. The unusually thick layer of primary spermatocytes appears when already produced spermatocytes cannot transform into spermatides because of lack of meiotic division. This process takes place under the control of FSH (SUN *et al.*, 1990), so the histological picture described above should be seen when relative or absolute FSH deficiency appears. Considering the time necessary to produce spermatozoons from spermatogonia (BOUSSIT, 1989) one should assume that the sudden drop of semen quality had to be caused by an agent that had started to work about 6 days before the disorder appeared. For the same reason this damaging factor could not have worked before meiotic division was complete, which means earlier than 16 days before the 11th of March. The only breeding accident that happened during this period was feeding with mouldy feed and it took place exactly between the 12th and 7th day before "disorder". Organoleptic examination of this feed suggested that it could have been infected with moulds (cakey, change of colour and smell).
The literature contains very few references to the influence of fungi infected feed and mycotoxins on reproduction system of mammals. It seems that the only mycotoxin that influences reproduction is zearelenone (F-2 toxin) produced by Fusarium sp. It does not provoke any clinical symptomes of intoxication, but proved to have very strong oestrogenic activity. Clinical cases of intoxication with feed containing F-2 toxin were described in pigs. Susceptibility of rabbits to this toxin was confirmed experimentally (JONES and HUNT, 1983). Unfortunately results of those experiments do not refer to histopathological picture of testis. Oestrogenic activity of zearalenone causes a disturbance of hormonal regulation of spermatogenesis. A very small quantity of oestrogens (E) is produced in the males' brain through the process of "aromatisation" of androgens

(testosterone T). They intervene in the negative control of the secretion of FSH. They are indeed very good suppressors of FSH release (MARTINI and ZOPPI, 1986). One should expect that exogenous oestrogens potentiate this suppressive activity. Consequently continuous application of exogenous oestrogens provokes continuous inhibition of FSH secretion. This in turn damages sustentacular cells, which results in thickening of basement membranes of seminiferous tubuli and spermiation of immature spermatozoons. Inhibition of FSH secretion also causes lack of meiosis in germinal epithelium.

On the other hand FSH deficiency makes interstitial cells insensible to LH activity. This relative LH deficiency is followed by a decrease in the intertubular tissue and number of interstitial cells, which was observed in the presented sections.

The feed in question was not checked for F2-toxin, but recent toxicological survey of Polish commercial feeds and concentrates showed the presence of this toxin in 3% of the examined concentrates (up to 1600 μg/kg) (JUSZKIEWICZ *et al.*, 1993). Another Polish survey concerning all kinds of cereals proved that zearalenon is present in 0,4% of the examined grains (200-700 μg/kg), especially in corn imported from Hungary, ex-Yugoslavia and France (JAMROZ, 1992).

Another well known source of phytoestrogens is Papilionaceae, but in Poland they are rather seldom used, so this probability is very low.

The presented hypothesis about F2-toxin as an agent disturbing hormonal regulation of spermatogenesis must be experimentally confirmed with special attention paid to histopathological changes in rabitt's testes.

Nevertheless one can already say that the lesions found in germinal epithelium of the examined animals are irreversible, because the scores of semen collected up to present day are still low (3-4) and insufficient to perform artificial insemination, while the same males covering naturally give 80-95% of gestations. It proves that the breeder must pay special attention to the quality of feed given to males, and not only to females.

REFERENCES

BOUSSIT D., 1989. Reproduction et insémination artificielle en cuniculture, 28-29. Ed. Association Francaise de Cuniculture.

JAMROZ D., 1992. Mikrobiologiczne skazenia pasz. *Polskie Drobiarstwo*, **I(2)**, 19-23.

JONES T. C., HUNT R. D., 1983. Diseases caused by Higher Bacteria and Fungi. *Veterinary Pathology*, 638-718. Lea and Febiger Ed., Philadelphia.

JUSZKIEWICZ T., KOZAK A., WISNIEWSKA-DMYTROW H., 1993. Skazenie mikotoksynami mieszanek paszowych i koncentratów w Polsce. *Medycyna Weterynaryjna* **49(6)**, 251-254.

MARTINI L., ZOPPI S., 1986. Mode of action of androgens in neuroendocrine structures. Andrology - Male Fertility and Sterility, 149-160. *Academic Press Inc.*