INFLUENCE OF TAMOXIFEN ON SEXUAL IMPULSE AND SEMEN BIOLOGICAL VALUES IN MALE DOGS

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Abstract

The study was conducted on 12 male dogs (5 to 10 years of age). The animals were divided into 2 groups: the experimental one (6 tamoxifen-treated dogs) and the control one (6 clinically healthy dogs). Besides, a clinical examination evaluating the overall state of health and sexual impulse, the examination of testosterone level in the blood serum was performed as well as the examination of semen (spermatozoa concentration, percentage of dead spermatozoa, evaluation of spermatozoa morphology, and evaluation of spermatozoa cell membrane activity – HOS test). It was found that tamoxifen had a negative influence on the function of the reproductive system in the dogs and caused, already in the first week of the treatment, the worsening of most of the evaluated characteristics of the semen and later, after the second week of the treatment, it produced aspermia with the complete loss of male fertility. The loss of fertility turned out to be periodical and lasted for about 60 d. After that time, the dogs regained the ability to produce ejaculate, though of much weakened quality, but later it was gradually improving.

Key words: dogs, semen, tamoxifen, spermatogenesis.

Cytostatics are most often toxic and their action is not only selective and limited to tumour cells, but also influences healthy cells and tissues of the organism (20, 27, 28, 30). The disadvantageous influence of cytostatics consists mostly in inhibiting functioning of the tissues, in which quick physiological processes of proliferation take place (9, 27, 30). These tissues belong to: marrow, lymphoid tissue, hair follicle cells, and epithelium cells of both ovaries and testicles.

In veterinarian practices in Poland, tumours in dogs and cats are diagnosed more and more frequently. Observations conducted in the Clinics of Animal Reproduction of the Agricultural University in Lublin as well as scientific publications indicate explicitly, a still growing number of tumour cases in the population of dogs and cats along with an increase in death rate for this reason (2, 29, 34). It is also a dominant cause of euthanasia in small animals (37). So far, tumour treatment in animals has been limited to the intervention of surgery, while chemotherapy was scarcely used. In recent years, especially in West European countries but also in Poland, veterinarians more frequently suggest chemotherapy in the treatment of both non-operative and operative tumours. In the latter case, anti-tumour preparations are used simultaneously with surgery in order to decrease the probability of metastases.

Material and Methods

The studies were conducted on 12 male mongrel dogs, 5–10 years of age, 15 to 36 kg of body weight (bw). The animals were physically fit and showed a proper sexual impulse, which was an absolute condition for choosing an animal for the experiment. The animals chosen for the studies underwent regular basic preventive and cosmetic treatment, such as against internal and external parasites, protective vaccination, bathing, ear cleaning, etc.

The animals were divided into two groups: control group consisting of 6 healthy dogs and the experimental group comprising of 6 dogs with clinically stated tumour changes qualified on the basis of present WHO TNM qualification (Tumour Nodes Metastases) to
the first or second group of the advancement of the tumour process. The animals were treated orally for 3 weeks with tamoxifen tablets (anti-oestrogen produced by Ratiopharm GmbH&Co.), at a dose of 2 mg/kg bw. The therapeutic dose was chosen on the basis of literature data (4, 21, 38). The treatment began 72 h after surgery conducted according to the common rules.

The animals of the experimental and control groups underwent the same procedure. It included everyday clinical examinations with special attention paid to the sexual system function, full semen evaluation, and examination of testosterone level in blood serum. The above-mentioned tests were conducted twice a day every fortnight before the drug treatment, thrice a day every 7 d during the treatment, and twice a day every fortnight after the treatment. Moreover, in the dogs of each group chosen randomly, histopathological testicle examination was performed during the treatment (on the 12th d) and on about the 50th d after the treatment. The period of examinations and observations in relation to each animal group, equalled at least 10 weeks and was conditioned by the necessity to follow the full cycle of spermatogenesis and spermiogenesis, which in dogs lasts for about 60 d. Because of consistent changes in semen quality, the observation was extended to 16 weeks.

Clinical examinations were aimed at the evaluation of the overall health condition of any particular animal in the group. An assessment of the sexual function of dogs was performed by the evaluation of the sexual impulse along with examination of accessible (external) reproductive organs. The evaluation of the sexual impulse was conducted in the presence of a bitch in heat. The libido was evaluated in a four-degree scale, from 0 to 4 according to the method described by England (10, 11).

Blood samples for the testosterone analysis was taken from the vena saphena into a silicone sterilized 9 ml tube (Vacuette, Greiner Labortechnik GmbH, Austria), and then centrifuged at 3 500 x g for 15 min, at 4°C. The received serum was stored at -78°C. The samples were taken 1 h after the i.v. injection of GnRH (Receptal–Interwet International B.V. Boxmeer, Holland) at the dose of 1 µg/kg bw (so-called GnRH stimulation test) (12, 20). The testosterone concentration was determined by radioimmunological method (RIA), using a commercial RIA kit (Orion Diagnostica, Finland). In this kit, the hormone was marked by iodine-I125. The sensitivity of the kit was below 0.1 nmol/L.

The semen for the examination was obtained by digital massage. The semen sample was instantaneously evaluated by routine methods (17, 39), which included: a) macroscopic evaluation – volume, consistence, colour, smell, potential additions, pH; b) microscope evaluation using Blom’s chamber – sperm mass motility, sperm individual motility, agglutination, density. The additional examination included: evaluation of the percentage of dead spermatozoon – after staining semen smears with eosin and nigrosin, evaluation of spermatozoon concentration by the cytometric method (on Bürker’s haemocytometer) and evaluation of spermatozoon morphological structure in fresh semen smears stained with gentian violet. The spermatozoon morphology was evaluated under a light microscope with a 1500x magnification, on the basis of Blom classification (3).

The biological activity of spermatozoon cell membrane was determined by a hypo-osmotic swelling test (HOS) recently introduced into diagnostics, in which spermatozoa are differentiated by their dependence on the cell membrane functional state. The HOS test was conducted in the following way: 1 ml of hypo-osmotic solution (fructose solution of osmolality 150 mOsm) heated to 37°C, mixed with 0.1 ml of the examined semen and incubated in a water bath at 37°C for 60 min. After the incubation, the sample was thoroughly mixed and about 10 µl of this mixture was placed on the heated slide glass with a glass cover. The evaluation of spermatozoa was performed under a light microscope with a heated stage, at 400x magnification. Two preparations were made from each semen sample. In each preparation, 100 spermatozoa were counted in a few different places and the percentage of spermatozoa with swollen head and bent tail was established (33).

From gonads removed during orchidectomy, segments were taken and preserved for 24 h in buffered 10% formalin of pH 7.2 and then embedded in paraffin blocks. Microscopic 4 µm thick preparations were stained with haematoxylin and eosin. At microscopic examination, attention was paid to the morphological structure of the testicles and epididymis, especially testicle interstitial cells, and the activity of seminiferous epithelium of seminiferous tubules.

Results of the studies are presented in a descriptive form and tables, graphs, and microphotos. The results presented in the tables were evaluated statistically by the Student’s t test with P≤0.05 considered as significant.

Results

In the clinical examination of the dogs, no visible deviations from the physiological state were found. In the experimental group (tamoxifen-treated), increased libido sexualis was observed. The increased sexual impulse was probably connected with the increased testosterone level in the blood serum of dogs during tamoxifen treatment (Fig. 1).

In Tables 1 and 2, the characteristics for the semen’s evaluation in the control and experimental groups of dogs were presented. The presented data revealed that the volume of ejaculate was significantly lower after the first week of tamoxifen treatment in comparison with the original volume, and in the following weeks of the treatment as well as at the end of examination (up to 14 weeks of observation) we failed to obtain any ejaculate. After 14 weeks, a very small volume of semen was again taken from the dogs (0.8 ± 0.75 ml) and this volume increased to 1.5 ± 1.12 ml in the 16th week of observation. All values received since
the 4th week of the experiment until its end were statistically lower in comparison to the control group.

The evaluation of individual spermatozoon morphology presented in table 1 revealed that already in the 1st week of tamoxifen treatment the percentage of spermatozoa was slightly lower (80.0 ± 6.32) in comparison with original values (83.3 ± 5.16). From the 5th to 14th week, aspermia was observed. After the subsequent semen collection in the 14th week of the experiment, only few spermatozoa showed proper individual motility (10.2 ± 6.08) and their number increased to 28.3 ± 4.17 in the 16th week of observation. The results received in the 14th and 16th week of the experiment were considerably lower than the physiological values, and were statistically significant in relation to the control group (P≤0.01).

Table 1 includes the percentage of dead spermatozoa in dog semen in the experimental group. The presented values revealed that already in the 1st week of tamoxifen treatment the percentage of dead spermatozoa was significantly higher than the original values. From the 5th to 14th week, aspermia was observed. In the 14th week most of spermatozoa were dead (72.5 ± 2.70); in the 16th week, the percentage of dead spermatozoa was still high (55.0 ± 5.20), but it decreased significantly in comparison with the previous examination. All values since the 4th week until the end of the observation were statistically higher (P≤0.01) in comparison with the control group.

The data on spermatozoon concentration in the experimental group revealed aspermia that lasted from the 2nd to 14th week of tamoxifen treatment. After such a long time, animals regained the ability to produce semen but spermatozoon concentration was very low. It was 5.4 ± 8.25 thousand/mm³ in the 14th week and it significantly improved in the 16th week of the observation (16.6 ± 5.50 thousand/mm³). However, it did not come back to the original state or to the physiological value. Differences in values in the 14th and 16th week of the observation in comparison with the control group were statistically significant (P≤0.01).

Spermatozoon morphology in the experimental group is also presented in Table 2. The data revealed that in the 1st week of tamoxifen treatment, the semen morphology did not change in comparison with the values before the treatment. From the 5th to 14th week of the observation, aspermia was observed. In the 14th week of the experiment the percentage of spermatozoa of the proper morphological structure was very small (13.7 ± 3.67), while defected spermatozoa constituted a very high percentage with major defects (56.7 ± 9.18) and minor defects (29.7 ± 7.74). In the 16th week of the observation, the percentage of defected spermatozoa in relation to correct spermatozoa was decreased. Values in the 14th and 16th week of the experiment were statistically significant.

Table 3 presents the results of the osmotic resistance test of dog semen in the experimental and control group. The data revealed that the percentage of spermatozoa reacting properly in the test (A) declined already after the 1st week of tamoxifen treatment (80.3 ± 3.10) in relation to the original values (88.8 ± 6.18), although it stayed within physiological norms. After regaining the ability to produce semen in the 14th week of the observation, the amount of spermatozoa with a functional cell membrane, that is reacting properly in the test, was very small (17.6 ± 4.26) in comparison with spermatozoa reacting negatively (82.4 ± 4.26). In the 16th week of the experiment the percentage of spermatozoa reacting properly increased considerably (63.1 ± 5.32). The values were statistically significant (P≤0.01) in the 4th, 14th, and 16th week of the observation.

The group of tamoxifen-treated animals showed very strong histological changes intensifying during the experiment. In the tamoxifen-treated animals (Fig. 2), seminiferous tubules often included immature, partially degenerated, and exfoliated cells of seminiferous epithelium. In testicle segments, the changes were even more intensive. Vast degenerative, necrotic, and atrophic changes of seminiferous epithelium up to the complete obliteration of its structure were observed. The lumen of most tubules was completely deprived of mature reproductive cells. A large part of seminiferous tubules contained only a single layer of cells consisting of spermatogonia and Sertoli cells. At the places where seminiferous tubules anastomose a focal hyperplasia of interstitial cells was observed. In focuses, where the cells underwent hyperplasia, an increased androgen dependent granulation in the cytoplasm was found. Spermatozoa were not noticed in the ducts of the epididymis.

Discussion

Tamoxifen, in generally accepted therapeutic doses for dogs (4, 21, 38), had a negative influence on the reproductive system of male dogs and caused periodical loss of fertility for about 60 days. These results confirm the findings of other authors who performed similar experiments on healthy dogs (13, 24), bulls (7), and rats (15, 35). The received results are; however, different from the data obtained in humans, who displayed the improvement of semen quality after tamoxifen treatment (22, 23). It should be noted that these experiments were performed on patients with oligozoospermia. Studies conducted by Salata et al. (23, 32) indicate the stimulating influence of tamoxifen on the defected spermatogenesis in humans and rats after previous oestrogen treatment. Thus, in the case of defected spermatogenesis, the increase of testosterone level after tamoxifen treatment causes proper spermatogenesis and therefore proper gonad function (19, 23).

Our studies were conducted on male dogs with a proper reproductive function, which could modify the received results. As there are very few publications concerning this problem in males, the mechanism of tamoxifen action responsible for periodical loss of fertility has not been precisely recognised.
Table 1
Investigated characteristics of sperm in dogs in experimental (tamoxifen treated) and control groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sperm sampling (weeks)</th>
<th>1</th>
<th>3</th>
<th>4</th>
<th>5-6</th>
<th>8-12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>During treatment</td>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejaculation volume (ml)</td>
<td>E</td>
<td>4.8±1.83</td>
<td>5.5±2.34</td>
<td>*2.2±0.95</td>
<td>asp.</td>
<td>asp.</td>
<td>**0.8±0.75</td>
<td>*1.5±1.12</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.3±2.06</td>
<td>4.2±1.72</td>
<td>3.6±1.03</td>
<td>4.2±1.47</td>
<td>4.2±1.43</td>
<td>4.0±1.67</td>
<td>3.8±1.47</td>
</tr>
<tr>
<td>Individual motility (%)</td>
<td>E</td>
<td>83.3±5.16</td>
<td>81.6±4.08</td>
<td>80.0±6.32</td>
<td>asp.</td>
<td>asp.</td>
<td>**10.2±6.08</td>
<td>**28.3±14.7</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>83.0±5.16</td>
<td>81.6±4.08</td>
<td>81.6±4.08</td>
<td>85.0±5.48</td>
<td>82.5±4.36</td>
<td>83.0±5.16</td>
<td>83.0±5.16</td>
</tr>
<tr>
<td>Dead spermatozoa (%)</td>
<td>E</td>
<td>4.3±2.16</td>
<td>3.2±1.33</td>
<td>**8.8±2.48</td>
<td>asp.</td>
<td>asp.</td>
<td>**72.1±12.4</td>
<td>**55.0±15.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.2±1.47</td>
<td>2.5±1.38</td>
<td>2.6±0.82</td>
<td>2.3±0.82</td>
<td>3.1±1.03</td>
<td>2.0±0.63</td>
<td>2.5±1.38</td>
</tr>
</tbody>
</table>

asp. – aspermia, E – experimental group, C – control group; NS – normal spermatozoon; MD – major defects; MND – minor defects;
* P≤0.05, ** P≤0.01.

Table 2
Characteristics of sperm in dogs in experimental (tamoxifen treated) and control groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sperm sampling (weeks)</th>
<th>1</th>
<th>3</th>
<th>4</th>
<th>5-6</th>
<th>8-12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>During treatment</td>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermatozoon concentration (n x10^6/cm³)</td>
<td>E</td>
<td>238.5±51.2</td>
<td>190.8±83.9</td>
<td>186.2±49.2</td>
<td>asp.</td>
<td>asp.</td>
<td>**5.4±2.54</td>
<td>**16.6±5.50</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>151.2±26.5</td>
<td>138.5±79.6</td>
<td>148.9±91.9</td>
<td>150.6±74.6</td>
<td>135.8±70.7</td>
<td>162.7±52.2</td>
<td>150.7±61.3</td>
</tr>
<tr>
<td>Morphology of spermatozoa</td>
<td>NS</td>
<td>85.1±3.94</td>
<td>88.7±4.13</td>
<td>88.3±6.10</td>
<td>asp.</td>
<td>asp.</td>
<td>**13.7±3.67</td>
<td>**73.0±5.46</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>86.4±5.34</td>
<td>90.3±2.60</td>
<td>88.1±3.92</td>
<td>90.1±3.34</td>
<td>88.5±2.21</td>
<td>89.2±2.06</td>
<td>89.5±1.73</td>
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<tr>
<td></td>
<td>MND</td>
<td>5.4±4.73</td>
<td>3.6±1.34</td>
<td>3.7±2.11</td>
<td>asp.</td>
<td>asp.</td>
<td>**56.7±9.18</td>
<td>**16.2±5.04</td>
</tr>
</tbody>
</table>

asp. – aspermia, E – experimental group, C – control group; NS – normal spermatozoon; MD – major defects; MND – minor defects;
* P≤0.05, ** P≤0.01.
Table 3
Results of osmotic resistance test of sperm obtained from experimental (tamoxifen treated) and control groups (%)

<table>
<thead>
<tr>
<th>Experiment time (weeks)</th>
<th>Before treatment</th>
<th>During treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Group</td>
<td>P</td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>E (n=6)</td>
<td><strong>87.3 ±2.25</strong></td>
<td>12.7 ±2.25</td>
<td><strong>88.8 ±6.18</strong></td>
</tr>
<tr>
<td>C (n=6)</td>
<td>88.5 ±5.58</td>
<td>11.5 ±5.58</td>
<td>85.3 ±3.98</td>
</tr>
</tbody>
</table>

Values are mean ± SD; E – experimental group (tamoxifen treated); C – control group, asp. – aspermia, N – negative reaction in osmotic resistance test (straight tail); P – positive reaction in osmotic resistance test (spirally rolled tail); * P≤0.05, ** P≤0.01.
Fig. 1. Testosterone level (ng/mL) in serum of dogs during experiment. *P ≤ 0.05, **P ≤ 0.01

Fig. 2. Seminiferous tubules. Degenerative and necrotic changes of seminal epithelium and its shaded structure. Local hyperplasia of Leydig cells. H – E staining, 160 x.
The first characteristic taken into account by a veterinarian evaluating a male for reproduction is his sexual behaviour, the so-called libido sexualis. Clinical observations conducted during tamoxifen treatment revealed that in some dogs the sexual impulse increased, what was evident in quicker and longer stimulation of males during semen collection. The increase of libido sexualis in males is probably the consequence of a significant increase in testosterone level in the blood serum in these animals. The high level of this hormone was observed during the whole period of tamoxifen treatment and it reached even 19.86 ng/mL. The received results are confirmed by other authors (23, 32), who also observed a significant increase in testosterone level during tamoxifen treatment in humans and rats. Lisowski et al. (22) treated roosters with tamoxifen and received similar results. Some authors showed that anti-oestrogen treatment, e.g. with cloimiphene citrate, significantly decreased the level of this hormone in bulls (28). The decrease of testosterone level after tamoxifen treatment was observed also by Sfikasis et al. (35). Gill-Shaema et al. (13) did not observe any influence of tamoxifen on testosterone level or on the reproductive function in male monkeys. Therefore, it could be stated that the mechanism of tamoxifen action is different in the particular animal species and even within the same species. The reason for the increase in testosterone level is probably the hyperplasia of Leydig cells responsible for the production of this hormone, which is evident in histopathological examination after tamoxifen treatment.

In the evaluation of ejaculate quality, many characteristics directly connected with the fertility of a dog reproductor were taken into account. The results received during the detailed semen study proved that tamoxifen negatively influenced most of the studied characteristics. It influenced the reproductive system in many stages. In the first week of the treatment the following disturbances were observed: decrease in semen quality, which was evident in its smaller volume, decrease in percentage of spermatozoa with proper individual motility, increase in dead spermatozoa percentage, and an increase of spermatozoa with the defective cell membrane (in HOS test). Semen evaluation was not performed in the second week of tamoxifen treatment due to aspermia in the treated dogs, which lasted for about 60 d, that is during the whole spermatogenesis cycle.

It should be noted that spermatogenesis in physiological conditions is a continuous process. In seminal ducts of male gonads, two processes take place simultaneously; one of them is leading to the continuous renewal of the constant number of parent cells and the other one is the process of multiplying and differentiating spermatogones into spermatocytes, which turn into spermatozoa (8, 11). Taking into account the observations made in the presented study; it should be assumed that tamoxifen blocks the continuous process of parent cell renewal and causes periodical lack of spermatogenesis. This is confirmed by histopathological examination of testicle segments of experimental dogs, where considerable atrophy and necrosis of the seminiferous epithelium was found. Other authors received similar results in dogs (38) and in rats (15, 35). The latter authors found that tamoxifen treatment at both low (1 mg/kg body mass) and high (10 mg/kg body mass) doses caused the decrease in testicle and alveolar gland mass. After the whole spermatogenesis cycle, the seminiferous epithelium started to recover and gradually the males regained the ability to produce semen, although at first the semen’s quality differed from the norms for fertile male ejaculate (11, 36). A significant decrease in semen volume and concentration, severe decrease in percentage of spermatozoa with proper individual motility, considerably increased necrozoospermia, defects of spermatozoon cell membrane, low percentage of spermatozoa with proper morphological structure, and higher percentage of spermatozoa with major and minor defects were observed in our experiment. These results are in accordance with the observations of other authors who showed the negative influence of tamoxifen on ejaculate quality (7, 22, 38). The characteristics of the semen’s quality and the amount of semen received in the 14th week of the experiment proved that the process of spermatogenesis began, but it was impossible to use a male for reproduction. However, in the last week of observation (16th week), all characteristics of the semen improved, which is the evidence of proper testicle function and spermatogenesis and spermiogenesis. Similar observations have been presented by other authors (38).

A precise recognition of the mechanism of tamoxifen action leading to the periodical lack of fertility in male dogs is not possible based on the methods used in this study. However, this was not the subject of the study. Yet it seems that one of the possibilities is the blocking influence of the oestrogen surplus, the so-called free - not linked with the oestrogen receptor, on the process of spermatogenesis. This state appears during tamoxifen treatment, which in this case acts as an oestrogen antagonist blocking the oestrogen receptors. In men with oligozoospermia, low testosterone level was found, and tamoxifen treatment caused its increase to the value allowing the proper spermatogenesis. Similarly in dogs, as was found by Kawakami et al. (19), low semen value was the result of low testosterone level. The proper biological semen value seems to depend on the proper correlation between the testosterone and oestrogen level (23, 32). In the studies that were conducted in men (23, 32) and in dogs (18) with the disturbed spermatogenesis process (oligo- or azoospermia), a significant increase in the oestrogen level was found, in comparison with physiological values. This confirms the negative influence of the surplus of these hormones on the fertility. The same authors stopped the process of spermatogenesis in rats by oestrogen treatment (23, 32). Kawakami et al. (18) demonstrated significant semen worsening in dogs with the increased oestrogen level. In our studies, the determination of the oestrogen level in the blood serum in experimental dogs was not performed, but taking into account the mechanism of tamoxifen action described by other authors (30); it could be assumed that also in experimental animals there was a significant
oestrogenisation, which stopped the process of production and differentiation of spermatozoa.

Another mechanism deflecting spermatogenesis is the influence of tamoxifen on the secretion of insulin-like growth factors IGF-I, and TGF, which as showed by many authors, have an essential influence on the proper course of spermatogenesis (1, 6, 14, 25). In vitro conditions, the growth hormone and IGF-I influence the process of steroidogenesis and the division rate of Sertoli and interstitial cells in rats and mice. The synthesis of IGF-I is also controlled by male steroid hormones as well as by the growth hormone. IGF-I is locally produced in testicles and plays an important controlling role in spermatogenesis cell division (quote after 25).

References


