

## **Клініко–морфологічні зміни артеріального кровотоку яєчка у дітей**

**В. М. Байбаков**

Дніпровський медичний інститут традиційної та нетрадиційної медицини

## **Clinical study of morphological changes in the arterial blood supply of the testicle in children**

**V. M. Baibakov**

Dnipro Medical Institute of Traditional and Non-Traditional Medicine

### **Реферат**

**Мета.** Вивчення клініко–морфологічних змін артеріального кровотоку яєчка за наявності його хірургічних захворювань і вдосконалення тактики хірургічного лікування.

**Матеріали і методи.** Проведено морфологічне дослідження 71 препарата яєчка і над'яєчка, отриманого під час автопсії у чоловіків віком 22 – 35 років, 38 препаратів оболонок яєчка за наявності його водянки, отриманих прижиттєво, а також як післяопераційний матеріал (порушення облітерації піхвового відростка очеревини), та 17 біоптатів яєчка, взятих у безплідних чоловіків, яким виконували хірургічні втручання на яєчках у дитинстві.

**Результати.** Використання інноваційного методу та впровадження в клінічну практику комплексної програми діагностики і хірургічного лікування захворювань яєчок у дітей дають змогу поліпшити кровообіг і знизити ризик ішемії яєчок.

**Висновки.** Клініко–морфологічні зміни артеріального руслу як ланки дренажних систем яєчка за наявності його хірургічних захворювань проявляються розвитком тотального судинного спазму та ішемії, обумовленим звуженням просвіту артерій, компенсаторною гіпертрофією м'язових шарів, потовщенням базальних мембран, що призводить до надлишку кровоносних судин, склеротичними змінами строми сім'яного канатика, деформацією сім'яносної протоки.

**Ключові слова:** артеріальний кровообіг; поєднане чоловіче безпліддя; дренажні системи яєчка; діти.

Abstract

**Objective.** Studying of clinical and morphological changes in the arterial blood supply of testicle in its surgical diseases and improvement of the surgical treatment tactics.

**Materials and methods.** In the morphological stage of the study, there was obtained the material from 71 preparations of testicle and epitesticle, which were taken while doing necropsy in men of a mature age (22 – 35 years old), also there were processed 38 preparations of membranes in dropsy testicle, taken in a life–time, as well as postoperative material (in non–complete obliteration of vaginal sprout of peritoneum), and 17 testicular biopsy specimens, obtained in infertile men, which were taken during surgical intervention on testis in pediatric patients.

**Results.** Introduction of the innovative method and implementation of complex program for diagnosis and surgical treatment of the testicular diseases in children into clinical practice have permitted to improve a testicular blood supply and to reduce a risk of the ischemia occurrence.

**Conclusion.** Clinical and morphological changes in the arterial blood supply as a part of drainage systems of the testicle in its surgical diseases are expressed in development of total vascular spasm and ischemia, which were caused by narrowing of the arteries lumen, compensatory hypertrophy of the muscle layers of arterial wall, thickening of basal membranes, leading to increase in the number of blood vessels, sclerotic changes in stroma of spermatic cord, deformation of the ductus deferens.

**Keywords:** arterial blood supply; combined male infertility; drainage systems of a testicle; children.

Pathological changes of testicular hemodynamics at the result of surgical testicular diseases in childhood are considered to be leading factor in the development of combined male infertility in the reproductive period [1, 2]. Pathogenesis of combined male infertility is complicated and insufficiently studied on a present stage, but it's associated with pathological changes all parts of drainage systems of the testicles [3, 4]. One of the leading factors in the development of male infertility is pathology of arterial blood stream, which appearing after surgical diseases of the testicle and surgical interventions [5 – 7]. Surgical diseases of the testicle, i.e. varicocele (element of a bypass renal anastomosis, which indicates about kidney vein obstruction or violation of outflow in the iliac vein with development of testicle ischemia), viola-

tion obliteration of vaginal sprout of the peritoneum (carried out to narrowing of arterial lumen, compensatory hypertrophy of muscular layers, thickening of basement membranes, closing a pathological circle, leading to the development of violations in all parts of drainage systems of the testicle), orchepididimitis (direct effect on fuel mediators on the spermatogenic epithelium or blood supply of the testicle with development of ischemia) are accompanied by total damage all parts of the drainage systems of a testicle [8 – 10]. These conditions should be grouped into one group, because its pathogenesis is based on the changes of the blood and lymph circulation of a testicle with further development violations of a germinal function [11 – 13]. Primary and pathogenetically grounded surgical correction for the given diseases is a vital

important step in order to prevent male infertility. It should be noted, that changes are more pronounced in bilateral lesions and their intensity increased with increasing terms of this disease without timely surgical treatment [14 – 16].

Results of treatment surgical diseases of the testicle in children, at the present stage, are not satisfying children's surgeons with a high percentage of complications. Firstly – it is a high risk of development male infertility in the reproductive age [17]. Therefore, solving of this problem is an important step in a struggle for reproductive health of nation and preservation of a full-fledged family [18, 19].

The data presented at work of L. Dumont et al. [20] will help to improve apoptotic and autophagic understanding during the first spermatogenic wave. Moreover, findings illustrate for the first time that, using finely-tuned experimental conditions, a testicular in vitro culture combined with proteomic technologies may significantly facilitate the study of cryopreservation procedures and in vitro culture evaluations.

On the manuscript of S. Rajanahally, et al. [21] were studied 30 pertained to marijuana and male infertility, 36 discussed cannabis and male sexual health/hormones, and 25 explored the relationship between marijuana and urologic neoplasms. With respect to male factor fertility using semen parameters as a surrogate, cannabinoids likely play an inhibitory role.

In the present study C. Meng et al. [22] found that L3MBTL2 was most highly expressed in pachytene spermatocytes within the testis. Germ cell-specific ablation of L3mbtl2 in the testis led to increased abnormal spermatozoa, progressive decrease of sperm counts and premature testicular failure in mice.

The data of A. E. El Zowalaty et al. [23] demonstrate novel roles of seipin in spermatid chromatin integrity, acrosome formation, and mitochondrial activity. Increased spermatid apoptosis, increased chromocenter fragmentation, defective chromatin condensation, abnormal acrosome formation, and defective mitochondrial activity contributed to decreased sperm production and defective sperm that resulted in Bsc12-/- male infertility.

M. Fode et al. [24] demonstrated, that low intensity extracorporeal shock wave therapy (Li-ESWT) may induce tissue regeneration, neo-angiogenesis and improve endothelial function. This has shown promise in the treatment of erectile dysfunction (ED). Li-ESWT has shown promise in pelvic pain and its effects on testicles have been preliminarily investigated in preclinical studies.

At the work of M. S. Rocca et al. [25] was shown that sperm telomere length (STL) is associated with standard semen quality parameters and it is significantly associated with levels of DNA fragmentation and sperm protamination. Telomeres are fundamental for genome integrity. Recent studies have demonstrated that STL increases with age and men with oligozoospermia have shorter sperm telomeres than normozoospermic men. Among standard semen parameters, STL was positively associated with progressive motility ( $p=0.004$ ) and vitality ( $p=0.007$ ). STL was significantly and negatively associated with sperm DNA fragmentation ( $p=0.001$ ) and significantly and positively associated with protamination ( $p=0.002$ ).

Purpose of the research: studying of clinical and morphological changes in the arterial blood stream of a testicle after its surgical diseases and improvement tactics of surgical treatment.

## Materials and methods

In the first, morphological stage of the study, was carried out material of 71 preparations of a testicle and epitesticle, which were taken after necropsy of men of the mature age (22 – 35 years), 38 preparations of the dropsy testicular membranes, taken in a life-time, as well as postoperative material (violations of obliteration vaginal sprout of the peritoneum), 17 testicular biopsy specimens of infertile men, which were carried out surgical intervention of testis in childhood (varicocele, violations of obliteration vaginal sprout of the peritoneum, acute testicle diseases). The Bioethics Commission on the protocol of the meeting of the Committee on Biomedical Ethics of the Dniprovskiy Medical Institute of Traditional and Alternative Medicine (No 1 from January 11, 2012) did not reveal violations of the moral and ethical norms during research work. For the normal state were accepted numerous literature reviews, concerning to the morpho-functional structure of a testicular drainage system in the different age groups.

In the second, clinical stage of the study, were represented 84 boys, which were undergo medical treatment in the surgical department of Municipal Establishment "Dnipro specialized clinical center of mother and child named by professor M. F. Rudnev" of Dnipro Regional Council" in a case of testicles hidrocele and inguinal hernia, predominantly at the age till 7 years – 75.2%. About 26 boys were treated varicocele, aged from 12 to 17 years. In a case of acute testicular diseases, on a basis of the surgical department were treated 39 boys, mainly at the age of 10 years – 87.3%. All children were treated for the period from 2012 to 2017.

Results of studies were recorded in the medical records of the patients and contained: complaints (presence of fluid formation in the scrotum or hernia stiffness, edema of the scrotum or presence of varicose veins in a scrotum on the left side), anamnesis (period of occurrence hidrocele or hernia, terms of edema in the scrotum), clinical data, ultrasound with a doppler examination of testicular vessels: index of resistance in the testicular arteries (RI), a peak systolic blood flow (Ps) and final diastolic blood flow (Md), method of surgical intervention.

## Results

Indicators of ejaculate in the combined male infertility were the following at the result of morphofunctional changes on the testicular drainage system: concentration of spermatozoa on million/ml –  $41.02 \pm 1.88$ , alive –  $44.03 \pm 2.07\%$ , active mobile –  $26.54 \pm 1.27\%$ , morphologically normal –  $6.24 \pm 2.21\%$ , pathology of a head –  $9.7 \pm 0.44\%$ , pathology of neck –  $15.46 \pm 0.74\%$ , pathology of tail –  $10.64 \pm 0.51\%$ , mixed pathology –  $18.49 \pm 0.86$ , pH –  $7.4 \pm 0.36$ , time of rarefaction –  $18.48 \pm 0.68$ . Content of fructose and citric acid in the ejaculate, as well as indirect indicator function of Leydig cells, was reduced. Volume of ejaculate was varied from 1.2 to 3.6 ml, pH – was in

the range 7.4 – 8.0. Number of spermatozoon in 1 ml:  $34.1 \pm 11.8$  million, among them alive –  $56.4 \pm 8.6\%$ , mobile –  $38 \pm 12.4\%$ , pathological forms –  $52 \pm 8.8\%$ .

*Orchoepididymitis.* Ultrasound examination of patients before and after surgery, carried out in the dynamics, allowed to assess presence of pathological changes in echogenicity, structure, volume of a testicle with epididymis and predict degree of violations of its drainage systems.

In the capsular testicular artery, both before and after surgery, was observed sharp decrease in a peak systolic blood flow velocity ( $V_{\max}$ ) and increasing of the resistance index. Such changes of blood circulation carried out immediately after surgery could be explained by operational trauma; compensatory increasing of blood circulation in the conditions of reconstruction of a vascular stream. Secondly, in the cases of significant decreasing number of vessels, vascular anastomosis, and network of the microcirculatory stream of a spermatic cord.

Stability of these indicators in a long term period of observation (in 6 months after conservative treatment of acute orchoepididymitis without a surgical intervention) was revealed in parallel with other negative characteristics, indicating about violation of damping mechanisms of protection, development of testicular hypoxia on the side of inflammation, restoration of blood vessels and network of a microcirculatory stream, but not in the full volume.

In the testicular artery after comprehensive treatment of inflammatory testicular disease in the 6 months after operation, was shown a significant improvement in the blood flow rates, compared with the state before operation. It was observed decreasing of the resistance index (0.607 – 0.600), increasing of a peak systolic blood flow and final diastolic blood circulation velocity (4.1 – 5.2 cm/s).

*Varicocele.* In this pathology, we observed significant morpho-functional changes in all parts of drainage systems of a testicle, which was manifested in the development of blood vessels plethora. There were developed sclerotic changes in the stroma of a spermatic cord, deformation of the ductus deferens. Lumen of arteries was narrowed due to compensatory hypertrophy of the muscle layers, thickening of basal membranes. In the capillaries was observed hyperemia and stasis.

*Violation of obliteration vaginal sprout of the peritoneum.* At the study of testicular circulation in children with abnormalities of a vaginal sprout of the peritoneum, was described increased index of resistance in the testicular arteries. Results of the studies focused on a presence of common disorders of arterial part of the drainage systems of a testicle with development of ischemia. Lumen of arteries was narrowed due to compensatory hypertrophy of muscle layers, thickening of basal membranes. In the capillaries was observed hyperemia and stasis. Taking into account anatomical features of location an arterial blood stream in the different levels of a spermatic cord, we proposed method of mobilizing vaginal sprout of the peritoneum in the local area – on the level of a deep inguinal ring. Traumatization of blood vessels on this level is minimal.

In 1–3 months after operation, ultrasound control with a doppler examination of testicular vessels was performed.

Testicular artery:  $RI=0.85 - 92$ ;  $V_{\max}=3.89 - 97$  cm/s. Indicators were practically corresponded to the normal for healthy children. Clinical and morphological signs of blood circulation disorders of a testicle were not observed.

Analyzing the obtained data, we concluded that in the testicular artery during implementation of the proposed method, which focused on the treatment of a vaginal sprout of the peritoneum on the level of a deep inguinal ring in children with complete disclosure of inguinal canal, after 24 months of surgical intervention, was shown improvement of the blood circulation, if compared before operation and among those patients, which undergone removal of a vaginal sprout of the peritoneum traditionally throughout a spermatic cord. It was shown reduction index of resistance (0.607 – 0.600), increasing of a peak systolic velocity of circulation (11.0 – 13.0 cm/s) and final diastolic velocity of blood (4.2 – 5.3 cm/s).

Obtained indicators were closely connected with the same indicators in healthy children ( $p < 0.05$ ). On the other side, indicators of the testicular lymphatic circulation after traditional intervention have been changed slightly, compared with the same indicators before surgery. On a basis of the obtained data, we concluded that proposed method is more effective than the traditional.

## Discussion

At the present stage is well-known that basic artery in vascular system of the testicle is testicular artery, because it's directly penetrates into the parenchyma of a testicle. From the testicular artery within a spermatic cord carried out artery of epididymis. Artery of the ductus deferens and artery of cremasteric muscle are involved into the blood supply of a testicle through anastomosis. The most common is anastomosis between the testicular artery and artery of ductus deferens. Diameter of a testicular artery varies from 0.2 to 1.9 mm, artery of the ductus deferens – from 0.2 to 1.8 mm, artery of the cremasteric muscle of a testicle – 0.1 – 1.5 mm. Diameter of testicle artery is equal or greater than the sum of other arteries more than in 50% of cases. According to studies, the internal vessels of a testicle are formed from two groups of arteries: those, which arise from artery of the testicle, penetrated to its parenchyma in a region of centrifuge arteries and those, which leaves from a vasculature, located under a protein membrane (central, radial arteries), which are also formed from the branches of testicle artery.

It is well-known, that in 80% of cases, artery of the epididymis penetrates into its capsule from a posterior–medial edge, separated on the artery of epididymis head and goes further along a medial edge, as a boundary artery of epididymis. Artery of epididymis could separate from artery of the ductus deferens. Thus, epididymis head having a blood supply from vessels of an upper pole of a testicle. Artery of cremasteric muscle of the testicle is placed between fibers of the cremasteric muscle in the 6 – 12 small trunks, which anastomosed in a tail section of the epididymis with marginal artery of the epididymis, or deferent duct artery.

*Orchoepididymitis.* Pathogenesis of infertility as a result of acute orchoepididymitis is associated, first of all, with chang-

es in a testicular vascular pathway. We proved that condition of a testicular drainage system affects on the spermatogram. In men with surgical diseases of the testicle was shown great pathological changes in the ejaculate, which caused by changes in the drainage systems of a testicle, such as oligozoospermia, azoospermia, akinozoospermia, teratozoospermia, necrozoospermia. Significant pathological changes at the beginning carried out in vessels of the arterial link, which is explained by total vascular spasm with development of ischemia. Further, in the vessels of arterial link was shown plethora, which becomes non-uniformly expressed, increased swelling of vessels, especially located under testicular membranes. Edema of a vascular wall appeared, which is changed by focal sclerosis and destruction of endothelium. Edema of connective tissue leads to the separation of tubes from vessels. External diameter of arterioles decreases, ischemia increases. In the blood vessels of microcirculatory stream defined a blood stasis, edema of a wall develops, basement membrane thickening, and endothelial cells exploded into the lumen. The vessel obliteration carried out.

Traditionally is considered, that treatment of patients with inflammatory of testicles, in most cases, should be conservative. It focused on the local use of daily novocaine blockages of spermatic cord, desensitizing therapy, suspension, antibacterial and anti-inflammatory therapy, physiotherapy. We propose, except conservative methods of treatment, carry out surgical treatment in any case of acute orchepididimitis, which should include scrototomy, revision of a scrotum and testicle. In our opinion, it is vital important to carry out, decompression of the testicle—cutting of the testicular membrane with required drainage cavity of the scrotum.

*Varicocele.* Lack of imagination about causes of varicocele and its harmfulness for the male reproductive function allowed surgeons to operate patients only in a case of severe pain in the scrotum. Under these conditions, results of the first methods of treatment of varicocele (searing of vessels, injection of sclerosing substances into vessels, resection of a scrotum, creation of an internal suspension, compression of enlarged veins by adjacent tissues) were not satisfy neither patients nor doctors. Problem of treatment varicocele was not only medical but also social, and needed to study complex of clinical and morphological investigations of vascular system of the testicle and spermatic cord. Effective and well-spread in the general clinical practice becomes the simple, safety and more effective "high ligation" operations. We prefer to suture a spermatic cord by Bernardy method (1941). This technique makes it possible to relate veins easily and invulnerably on the level of a deep inguinal ring in the intraperitoneal space.

Operative intervention should be performed with cut of skin on 2 – 3 cm above the projection of a deep inguinal ring, parallel to the inguinal bundle. After the muscles separation, testicles of the peritoneum are visualized. Testicle veins are carefully allocated, dressing proximally and distally, and dissected. Wound of anterior abdominal wall is stitched up by layers.

*Example.* Patient M. Maxim, 15 years old, was hospitalized to the surgical department of Municipal Establishment "Dni-

pro specialized clinical center of mother and child named by professor M. F. Rudnev" in Dnipro Regional Council" on 22.11.2013 year. Parents of the child complaints on branched out as a rod protrusion in the left side of a scrotum. At the examination of inguinal–scrotum area on the left side, was confirmed the branched out as a rod protrusion 30x45 mm size, which retained its size in standing and lying position. The testicles were in a scrotum. Diagnosis: varicocele on the left side III degree. Surgical intervention was recommended.

After preparation under general anesthesia, was carried out a layered opening of tissue on 2 – 3 cm above projection of a deep inguinal ring, parallel to the inguinal bundle. After the muscles separation, into the surgical wound were removed two dilated on 0.8 – 1.0 cm in diameter testicular veins, which were placed on the peritoneum. These vessels were carefully selected, proximal and distal vessels were dressing, then dissected. Wound of the abdominal cavity was tightly sutured by layers. After three months of the operation was performed an ultrasound with doppler examination of testicular vessels on the left side. Testicular artery: RI=0.89; Vmax=3.89 cm/s. Disorders of the blood circulation of a testicle were not observed. Venous blood circulation of the left testicle has high amplitude, corresponded to the respiratory waves, having a clear phase, which caused by restoration of a vascular elasticity. Results of the studies indicated about a lack of circulatory disorders.

*Violation of obliteration vaginal sprout of the peritoneum.* We have been worked on the improvement of method of surgical treatment a vaginal sprout of peritoneum. There were performed 84 operations in the children. Among them 42 operations were performed by the traditional method, with complete removal of vaginal sprout of the peritoneum through-out a spermatic cord, starting from the level of entrance into inguinal canal without its disclosing. For another 42 patients, vaginal sprout of the peritoneum was crossed over a local region, on the level of a deep inguinal ring after full opening of an inguinal canal throughout its length. Treatment of the testicles at the hydrocele in all cases was carried out traditionally, by Ross method.

Operative intervention by author's method began with access in the inguinal area. Layer by layer an anterior abdominal wall was cut to the aponeurosis of external slanting skeletal muscle of abdomen. Front wall of inguinal canal was cut such way in order to put free edge of the external slanting skeletal muscle of abdomen into the wound and to visualize a deep inguinal ring. By Farabeff' hooks were raised free edge of the external slanting skeletal muscle of abdomen and the lumbar abdominal muscles. A spermatic cord was mobilized on the local site, in the level of a deep inguinal ring after hydraulic preparation with physiological solution of sodium chloride, and continued it's removing with a full intersection across a vaginal sprout of the peritoneum. Traumatization of lymphatic and blood vessels on this level is minimal. Aponeurosis of the external slanting skeletal muscle of abdomen was sutured with the formation of duplicate.

Implementation model of the surgical treatment of varicocele in children into the clinical practice should contribute

further development means of treating surgical diseases of the testicle and a spermatic cord, normalizing of the physiological and psychological development of the child.

In the future, using of this program should reduce risk of developing of the male infertility in a reproductive period.

Obtained results testify about negative influence of varicocele, acute inflammatory diseases of a testicle and violations obliteration of a vaginal sprout of the peritoneum to the morpho-functional state not only an arterial, but all parts of the drainage systems of a testicle. Vital necessity measures should be carried out on the timely implementation of pathogenically grounded surgical intervention in the surgical pathology of a testicle in children.

Using of innovative method and implementation to the clinic of the complex program for diagnosis and surgical treatment of the testicular diseases in children would improve testicular circulation and reduce risk of ischemia.

Results of research give an opportunity to decrease development of complications in a practice as well as pathological process itself and risk of development of the postoperative complications.

## Conclusions

1. Clinical and morphological changes of arterial stream as a link of drainage systems of the testicle with its surgical diseases are expressed in the development of total vascular spasm and ischemia, which caused by narrowing of arteries lumen, compensatory hypertrophy of the muscle layers, thickening of basal membranes, leading to the plethora of blood vessels, sclerotic changes in a stroma of spermatic cord, deformation of the ductus deferens.

2. Results of operational correction of surgical diseases of a testicle in children confirmed relevance and high efficiency of the proposed complex program for diagnosis and treatment on a basis of clinical data and ultrasound with doppler examination of the testicular vessels. The given findings focused on the decreasing of a resistance index (0.607 – 0.600), increasing of a peak systolic blood circulation velocity (11.0 – 13.0 cm/s) and final diastolic blood circulation velocity (4.2 – 5.3 cm/s).

3. It had been proved, that primary and pathogenically grounded surgical treatment of varicocele, acute inflammatory diseases of a testicle, abnormalities of vaginal sprout of the peritoneum in childhood should be an important link in prevention of male infertility.

## Acknowledgements

**Financing.** At own expense.

**Conflict of interest.** The author, who have taken part in this study, declared that, they do not have any conflict of interest with respect to this manuscript.

## References

1. Gozdas HT, Bal T. Brucellar epididymo-orchitis: a retrospective study of 25 cases. *Aging Male*. 2019 Feb;15:1–4. doi: 10.1080/13685538.2019.1573892.
2. Zhang FP, Malinen M, Mehmood A, Lehtiniemi T, Jääskeläinen T, Niskanen EA, et al. Lack of androgen receptor SUMOylation results in

- male infertility due to epididymal dysfunction. *Nat Commun*. 2019 Feb; 10(1):777. doi: 10.1038/s41467-019-08730-z.
3. Gärtner SMK, Hundertmark T, Nolte H, Theofel I, Eren-Ghiani Z, Tetzner C, et al. Stage-specific testes proteomics of *Drosophila melanogaster* identifies essential proteins for male fertility. *Eur J Cell Biol*. 2019 Jan 17. pii: S0171-9335(18)30268-1. doi: 10.1016/j.ejcb.2019.01.001.
4. Cathro HP. Iatrogenic Disease of the Genitourinary Tract. *Adv Anat Pathol*. 2019; Feb 1. doi: 10.1097/PAP.0000000000000226.
5. Jacobo P. The role of regulatory T Cells in autoimmune orchitis. *Andrologia*. 2018 Dec; 50(11):13092. doi: 10.1111/and.13092.
6. Fode M, Russo GI, Verze P. Therapeutic areas of Li-ESWT in sexual medicine other than erectile dysfunction. *Int J Impot Res*. 2019 Jan 22. doi: 10.1038/s41443-019-0114-2.
7. Zhang FP, Malinen M, Mehmood A, Lehtiniemi T, et al. Lack of androgen receptor SUMOylation results in male infertility due to epididymal dysfunction. *Nat Commun*. 2019 Feb;10(1):777. doi: 10.1038/s41467-019-08730-z.
8. Zhang Y, Zhang Y, Zhang L. Expression of cancer-testis antigens in esophageal cancer and their progress in immunotherapy. *J Cancer Res Clin Oncol*. 2019 Feb;145(2):281–291. doi: 10.1007/s00432-019-02840-3.
9. Mallé M, Coulibaly AM. Voluminous unilateral hydrocele with scrotal ulceration: about a case study conducted at the Regional Hospital of Gao. *Pan Afr Med J*. 2018 Aug 7;30:257. doi: 10.11604/pamj.2018.30.257.16373.
10. Zhou SH, Deng YF, Weng ZW, Weng HW, Liu ZD. Traditional Chinese Medicine as a Remedy for Male Infertility: A Review. *World J Mens Health*. 2019 Jan 10. doi: 10.5534/wjmh.180069.
11. Shoorei H, Khaki A, Khaki AA, Hemmati AA, Moghimian M, Shokohi M. The ameliorative effect of carvedilol on oxidative stress and germ cell apoptosis in testicular tissue of adult diabetic rats. *Biomed Pharmacother*. 2018 Dec;111:568–578. doi: 10.1016/j.biopha.2018.12.054.
12. Huang Y, Shi X, Zhong P, Wang Y, Xiao H, Zhou X, Yun J. De Novo Testicular Extranodal NK/T-Cell Lymphoma: A Clinicopathologic Study of 21 Cases With Review of Additional 18 Cases in the Literature. *Am J Surg Pathol*. 2018 Dec 26. doi: 10.1097/PAS.0000000000001210.
13. Wang LL, Li ZH, Duan YG, Yuan SQ, Mor G, Liao AH. Identification of programmed cell death 1 and its ligand in the testicular tissue of mice. *Am J Reprod Immunol*. 2018 Dec 22:e3079. doi: 10.1111/aji.13079.
14. Chovanec M, Albany C, Mego M, Montironi R, Cimadamore A, Cheng L. Emerging Prognostic Biomarkers in Testicular Germ Cell Tumors: Looking Beyond Established Practice. *Front Oncol*. 2018 Nov 28; 8:571. doi: 10.3389/fonc.2018.00571.
15. Kalavska K, Kucerova L, Schmidtova S, Toro L, Kozovska Z, Plank, et al. Lymphoma transformation of tumor infiltrating lymphocytes observed in testicular patient-derived xenograft models. *Oncol Rep*. 2018 Dec;40(6):3593–3602. doi: 10.3892/or.2018.6769.
16. Simard M, Laprise C, Girard SL. Impact of Paternal Age at Conception on Human Health. *Clin Chem*. 2019 Jan; 65(1):146–152. doi: 10.1373/clinchem.2018.294421.
17. Wang LL, Li ZH, Duan YG, Yuan SQ, Mor G, Liao AH. Identification of programmed cell death 1 and its ligand in the testicular tissue of mice. *Am J Reprod Immunol*. 2018 Dec 22:e13079. doi: 10.1111/aji.13079.
18. Bhanmeechao C, Srisuwatanasagul S, Prapaiwan N, Ponglowhapan S. Reproductive aging in male dogs: The epididymal sperm defects and expression of androgen receptor in reproductive tissues. *Theriogenology*. 2018 Mar 1; 108:74–80. doi: 10.1016/j.theriogenology.2017.11.011.
19. Ferlin A, Rampazzo E, Rocca MS, Keppel S, Frigo AC, De Rossi A, Foresta C. In young men sperm telomere length is related to sperm number and parental age. *Hum Reprod*. 2013 Dec; 28(12):3370–6. doi: 10.1093/humrep/det392.
20. Dumont L, Chalmel F, Oblette A, Berby B, Rives A1,2, Duchesne V, et al. Evaluation of apoptotic- and autophagic-related protein expressions before and after IVM of fresh, slow-frozen and vitrified pre-pubertal

- mouse testicular tissue. *Mol Hum Reprod.* 2017 Nov 1;23(11):738–754. doi: 10.1093/molehr/gax054.
21. Rajanahally S, Raheem O, Rogers M, Brisbane W, Ostrowski K1, Lendvay T, et al. The relationship between cannabis and male infertility, sexual health, and neoplasm: a systematic review. *Andrology.* 2019 Feb 15. doi: 10.1111/andr.12585.
22. Meng C, Liao J, Zhao D, Huang H, Qin J, Lee TL, et al. L3MBTL2 regulates chromatin remodeling during spermatogenesis. *Cell Death Differ.* 2019 Feb 13. doi: 10.1038/s41418-019-0283-z.
23. El Zowalaty AE, Baumann C, Li R, Chen W, De La Fuente R, Ye X. Seipin deficiency increases chromocenter fragmentation and disrupts acrosome formation leading to male infertility. *Cell Death Dis.* 2015 Jul 16; 6:e1817. doi: 10.1038/cddis.2015.188.
24. Fode M, Russo GI, Verze P. Therapeutic areas of Li-ESWT in sexual medicine other than erectile dysfunction. *Int J Impot Res.* 2019 Jan 22. doi: 10.1038/s41443-019-0114-2.
25. Rocca MS, Speltra E, Menegazzo M, Garolla A, Foresta C, Ferlin A. Sperm telomere length as a parameter of sperm quality in normozoospermic men. *Hum Reprod.* 2016 Jun; 31(6):1158–63. doi: 10.1093/humrep/dew061.