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Round Immature Spermatogenic Cells in Semen Fluids of Infertile Men with Diagnosis "Migrating Testis". Two Casuistic Cases in Adults

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The spermatological analysis of ejaculates from two patients suffering of low male fertility and with diagnosis "migrating" testis syndrome (casuistic cases in adults), reveals cytological characteristics of different immature spermatogenic cells ("round cells") as precursors of spermatozoa. The high quantity of undifferentiated spermatocytes/spermatides in ejaculates is important for early diagnoses of andrological diseases related to male fertility (sub-fertility, infertility). The results from semen assays showed a high percent (7.3% and 13.5%) of immature ("round") spermatogenic cells in the ejaculates of patients – in correlation with the elevated number of abnormal spermatozoa in probes. The morphological analysis of sperm samples in patients with ascending testis could serve as additional diagnostic and prognostic tool in the routine everyday andrological practice.

Key words: spermatogenesis, immature spermatogenic cells ("round cells"), ascending testis syndrome.

Introduction

In the process of spermatogenesis, spontaneous degenerative changes in the separated zones of the seminiferous tubules are possible. These features affect individual cell types – spermatogonia, spermatocytes (primary and/or secondary), spermatids and spermatozoa, and some of them pass in the seminal fluid. In pathology of the genital tract, which could be a result of congenital factors (cryptorhidism, anorhidism, etc.) or of the influence of different diseases, these changes can include larger regions of the testicular tissue [7, 14, 20]. In normal conditions, the amount of the degenerated gametes in the ejaculate is approximately 25% (WHO, 2010). However, in stress conditions, significant increase is possible, which could influence the inseminal qualities of the fluid. The morphological assessment of the seminiferous assay includes the determination of the mature spermatozoa, but also of other – both cytologically and functionally different

cell types. In most cases these are immature germ cells – precursors of spermatozoa, but also leucocytes (e.g. granulocytes, monocytes/macrophages) or epithelial cells from the urogenital tract. The immature germ cells, together with the leucocytes, usually are beyond the general group of so called "round cells" [4, 11, 14]. The exact determination of the cell types and their differentiation in spermatogenic or non-spermatogenic mature cells is important not only for a correct diagnosis, but also for the therapeutic approach. According to the recommendations of the World Health Organization (WHO), if the "round cells" in the ejaculate probe are more than 1×10^6/ml, they should be determined as "presenting leucocytes", because their increased amount could be a sign for inflammation process in the urogenital tract [6, 19]. In this connection, it is of interest to study the cytological content of the seminal fluid in patients with diagnosis "migrating" testis – two casuistic cases in adult men. In the scientific literature, the term "migrating" (elevator) testis ("retractile tests") [2, 12, 23] is also described as a "retractile testis" and/or as "a syndrome of ascending testis" [12, 20, 23]. This abnormal gonad state is usually diagnosed in boys (child and juvenile age – most often around 10 years), but it is extremely rare in adult men. Unlike in cases of cryptorhidism, despite the scrotal position of the testes there is a risk of migrating testes (they remain in inguinal duct and later descend into the scrotum) due to external conditions like cold, nerve stress of cremaste muscle. As a result, abnormalities in the spermatogenesis are possible, which are similar to those, established in diagnosed cryptorhidism and/or varicocelle, due to the abnormal gonad thermoregulation [20, 23].

In this connection, the aim of the present study is to evaluate the quantity and to characterize the morphology of "round cells" in the samples from semen fluids of patients with more rare diagnosis *ascending testis*. The methods of qualitative and quantitative semen analysis, applied by us, after *in situ* staining of "round cells" in ejaculates, could be an important diagnostic tool applicable in the treatment of patients with disturbances in spermatogenesis and subfertility/infertility-related problems.

Materials and Methods

Semen liquids (ejaculates) from two andrological patients (average 23 and 30 years) with *ascending testis*, are examined, and the results are compared with data, obtained from a control group of 20 fertile healthy men (average 32.45 ± 1.59 years).

For a precise clinical diagnosis, in each case the patient's data (anamnesis), together with his local clinical status, are carefully investigated.

Sperm Analysis

Spermatological assay includes the qualitative and quantitative analyses of ejaculates and investigations of individual cells (spermatogonia, spermatocytes, spermatids, spermatozoa and other, so-called "round cells", in seminal plasma – assessed according to WHO criteria, 2010).

For cytological analysis, the smears from ejaculates are stained by the standard methods of Papanicollaou and Hematoxyllin/Eosin. The slides are examined light-microscopically with microscope Leica DM 5000B (at different magnifications).

On the basis of morphological characteristics, the spermatogenic and non-spermatogenic ("round") cells in ejaculates are identified, as:

- spermatogonia (Spg);
- primary spermatocytes (Spc-I);
- secondary spermatocytes (Spc-II);
- spermatids (Spt);

– round spermatids – rSpt;
– elongated spermatids – elSpt;
- other (non-spermatogenic) round cells:
  – granulocytes;
  – monocytes/macrophages;
  – lymphocytes.

Statistical Analysis

Statistical significance is verified by Student's *t*-test. The results are given as MEAN ± SD.

Results

In the course of the spermatological analysis we obtained a reduced number of morphologically normal spermatozoa in the ejaculates of two andrological patients (with ascending testis), in comparison with the normal values for the control group. The quantitative results are presented in **Table 1**.

Table 1. Cell number and presence of spermatozoa (including abnormal), as well as undifferentiated (immature) spermatogenic cells in ejaculates of patients with *ascending testis*, versus data for the control group

	Number of spermatozoa (million/ml)	**Normal spermatozoa (%)**	**Abnormal spermatozoa (%)**	**Immature germ cells (million/ml)**	**Immature germ cells (%)**
Ascending testis (n = 2)	(n = 1) Normospermia 67	38.3	54	5.59	7.3
	(n = 1) Oligospermia 19	22.95	63.45	2.97	13.5
Control group (n = 20)	84.79 ± 13.57	80.63	18.37	0.86 ± 0.09	1.0

In one of the patients, the results show *oligospermia* – significantly decreased total spermatozoa number (19 million/ml) in the ejaculate, and in the other – *normospermia* (67 million/ml). In both cases, especially in that with *oligospermia* we assessed increased (> 50%) percent of the spermatozoa with abnormal morphology (**Table 1**). The highest percent is established for the spermatozoa (18.46%), possessing deformations in the head, but gametes with combined abnormalities also reach significant percent (25.71%) (**Fig. 1**).

According to our results, simultaneously with the decrease of total number of spermatozoa, an increase in the percent of the spermatogenic "round cells" in the ejaculates was found, which correlates with the higher percent of the abnormal gametes. The percentage of the immature "round" spermatogenic cells in the samples of both patients is

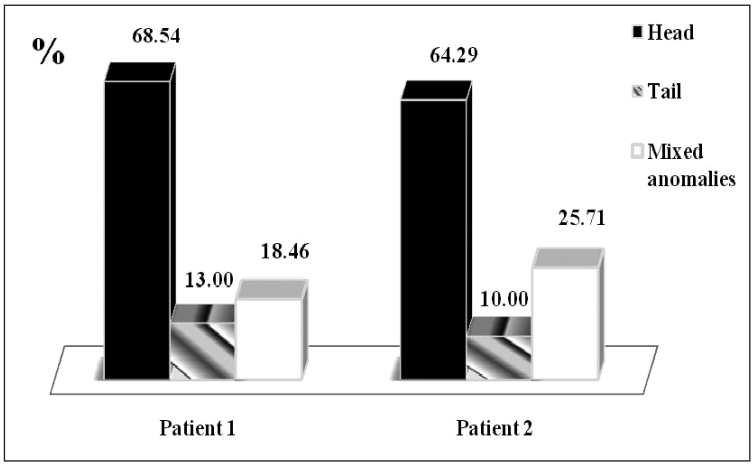

Fig. 1. Percent (%) distribution of the morphological spermatozoa abnormalities (in head, tail, mixed anomalies) in the two patients with ascending testis (Patient 1 – *normospermia*, Patient 2 – *oligospermia*)

significantly increased (7.3% and 13.5%, respectively), in comparison with the control group (1%). In **Table 2** is presented the percent distribution of the morphologically immature spermatogenic cells on cytological smears from the patients with "ascending testis".

Table 2. Percent (%) distribution of immature spermatogenic cells in the ejaculates of patients with "ascending testis" (Patient 1– *normospermia*, Patient 2 – *oligospermia*)

%	Spg	Spc-I-II	Spt
Patient 1	3.6	20.7	75.7
Patient 2	3.7	21.4	74.9
Control group (n = 20)	–	6.8	93.2

The distribution in **Table 2** shows higher percent of the spermatids in all cases, but unlike in the control group, in both patients we established increased number of spermatogonia, and spermatocytes. In all ejaculates tested, the leucocytes amounts vary (0:0.4%).

The microscopical investigations of semen fluids resulted in a precise morphological characterization and identification of spermatogenic cells at different stages of cell maturiration/differentiation: from undiferentiated spermatogonia to mature spermatozoa (**Figs. 2, 3**).

Spermatids (with round or oval nuclear shape) and spermatocytes are the most often spermatogenic "round cells" identified in the ejaculates of patients with "ascending testis" (**Fig. 2**).

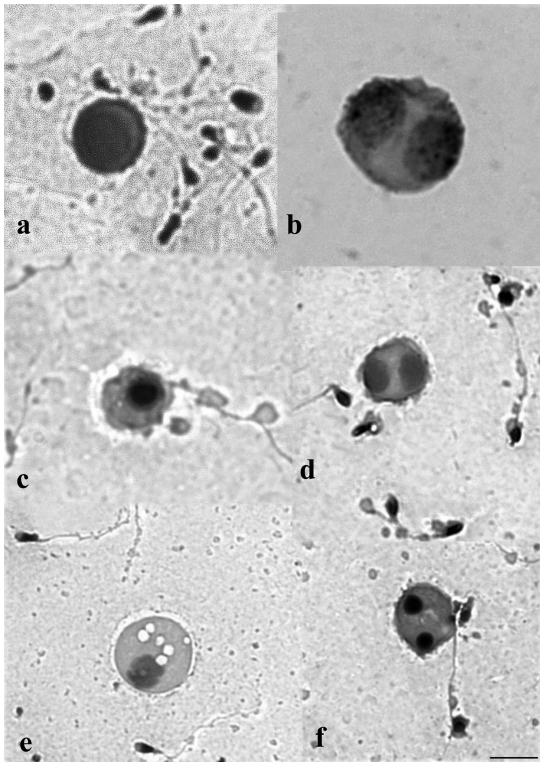

Fig. 2. Spermatocytes and spermatids: a) Spc I; b) Spc I with two nuclei; c) round Spt; d) Spc II with two nuclei; e) degenerating Spt with vacuoles in cytoplasm; f) degenerating Spt with two picnotic nuclei. Papanicolaou (× 400; × 600)

Spermatogonia and secondary spermatocytes are also found, but much seldom than the other spermatogenic cell types. A common morphological feature in the ejaculates of these patients is the high amount of primary (Spc I) and secondary spermatocytes (Spc II), possessing two nuclei, probably in result of abnormal mitoses (**Figs. 2b, d, f**).

The presence of similar round cells, but with larger size, possessing four nuclei, (probably spermatocytes), is more often observed in the patient with *oligospermia* than in that with *normospermia* (**Figs. 3d, 3e**).

Multinuclear cells, containing two, three or more picnotic spermatide nuclei, are also established (**Figs. 3a, c**), as well as the presence of groups (clusters) of cells or couples of cells, connected to each other by intra-cellular links ("cytoplasmic bridges") (**Figs. 3g, h**).

Another often met finding is the presence of large round cytoplasmic residues (cytoplasmic droplets), around the mature spermatozoa, with or without agglutination. Similar accumulation of mature spermatozoa is also established around the round immature germ cells (**Figs. 3f, i**).

The morphological characterization of the most spermatids (with round or oval shape of the nucleus) illustrates a high degree of male gamete degeneration, associated with injured nuclear chromatin condensation and cytoplasm vacuolization (probably due to apoptotic changes), (**Fig. 2e**).

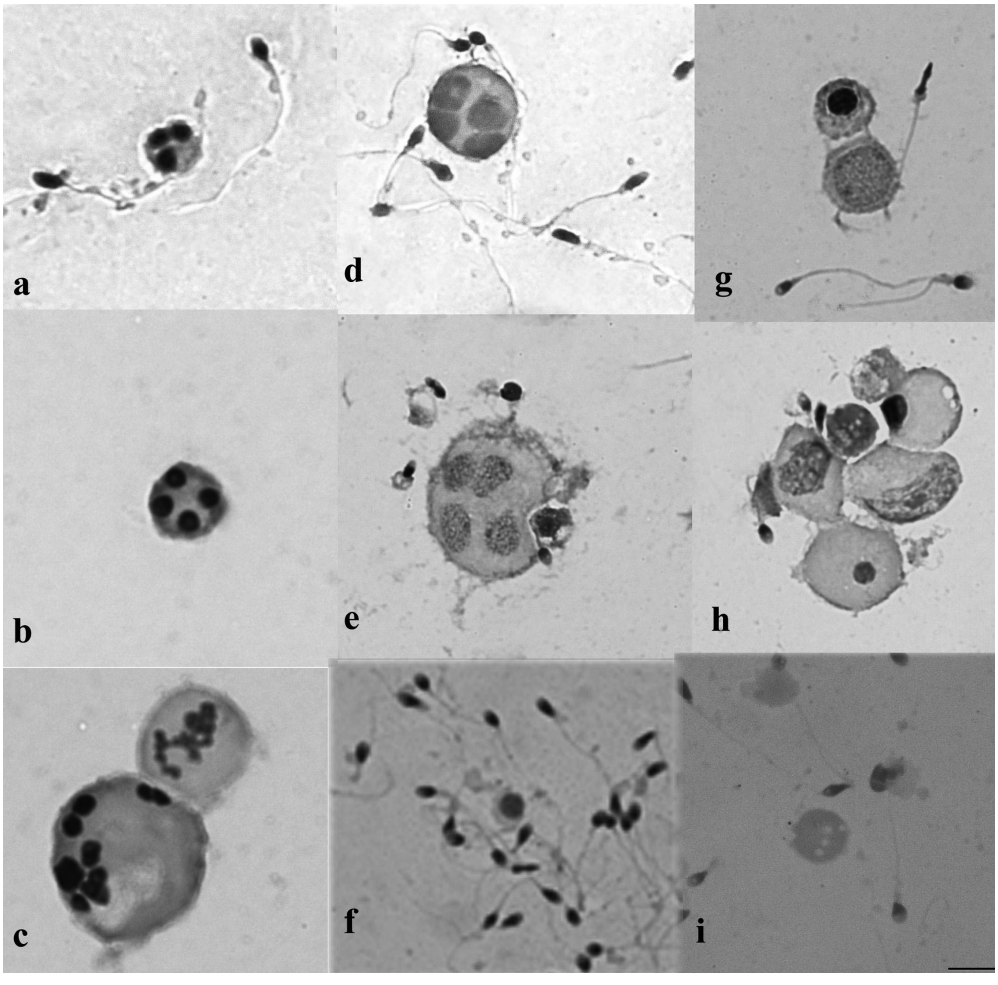

Fig. 3. Round cells and clusters: multi-nuclear cells (a – c) with picnotic nuclei (d – e); with four nuclei (non-finished cell division) (Spc); (f) Spc I and agglutination; (g – h) couple of cells and group (cluster) of cells; (i) cytoplasmic remedy (droplet). Papanicolaou, HE (× 400; × 600)

The spermatological assays of the ejaculates from both patients demonstrated a decreased seminal ability with sub-fertility in the case with *normospermia*, and infertility in *oligospermia*, respectively.

Discussion

Undescended testis or cryptorchidism represents the most common congenital abnormality in boys. The main reason of infertility in cryptorchidism is hyperthermia related to the abnormal position of testis that impaired spermatogenesis [16]. From the clinical (andrological) and pathological point of view the ascending (migrating) testis demonstrates similar features to these of cryptorhidism – with impaired gonadal thermo-regulation and abnormal spermatogenesis, falling by this way into the categories of "similar

diseases" [13, 14, 20]. If these disorders are not treated for a prolonged time period, they could lead to an increased risk for infertility, but also to development of testicular tumors in adults [3, 5].

In the current spermatological study, a high percent of immature spermatogenic cells in the ejaculates of both patients with "migrating testis" diagnosis, is established – 7.3% and 13.5%, respectively. Although we obtained results from two patients only, the increased amounts of spermatids and spermatocytes in the seminal probes might be due to pathological seminiferous tubules. The observed groups (clusters) of two, three or more cells, connected to each other by cytoplasmic connections ("bridges"), but also the presence of two- and multi-nuclear spermatids (most often with picnotic nuclei), show an interesting cytological pattern in both *oligospermia*- and *normospermia*-diagnosed patients. In both cases, the reduced inseminal possibility is associated with the increased percentage of abnormal spermatozoa, in particular such with deformations in the head, as well as with mixed anomalies. These results are similar to our previous data from investigations of patients with pathologies of the male reproductive system [9, 10], where we also established increased amount of gametes with head anomalies. In cases with *cryptorchidism* the spermatozoa with elongated and round heads were prevailed, but increased number of cells with two heads was also observed. However, in comparison with the patients with ascending testis, significantly lower amount of the gametes with mixed anomalies was assessed in the cases with *cryptorchidism*.

According to many authors [8, 20, 22], the results from histological studies indicate that the spermatogenesis could be affected and injured in all stages of the male reproductive system development (ontogenesis, puberty and adult age). Different etiological factors could lead to the same and/or to similar structural abnormalities in the testicular tissue, manifested by decreased germ cells proliferation, abnormal differentiation and hence appearance of "teratological forms" among the mature spermatozoa as well as elimination of many immature spermatogenic cells. The gametogenesis abnormalities in cases with *cryptorhidism*, are often characterized by blocking of the process at the different phases of spermatocytes and/or spermatids' development in the different regions of the seminiferous ducts. The blocking is depending on the susceptibility of the respective cell populations to the increased temperature in the scrotum, as well as to the subsequent hypoxia and oxidative stress [3, 14, 18]. Depending on the pathological conditions and factors, however, changes in the spermatogenesis are possible not to occur or they could be in non-equal degree in all tubules, and intact spermatogenesis might also be established. On the other hand, the changes could be transitive, in non-constant hypospermatogenesis, associated with intra-tubular disorganization, suppression and/or arrest of the germ cells maturation process. Moreover, changes could be definitive, characterized by germinal aplasia, tubular sclerosis and/or progressive peritubular fibrosis in the testis, leading to *azoospermia* [1, 17]. In the patients with *ascending testis*, the cytological observations suggested cell apoptosis activation in early stages of germ cells development (depending on the time period of the gonads in hyperthermia conditions), rather than a concrete affection in any spermatogenic stage.

The established in the current study high percentage of abnormal spermatozoa, as well as in other disorders of the male reproductive system, suggests altered function of the *epididymis*, responsible for further proceses of the spermatozoa capacitation, and acquisition of motility. The defects in the spermatozoa formation are often connected with injuries in the cellular DNA and nuclear chromatin structure [15, 24], important in application of technologies for *in vitro*-insemination.

Assisted reproduction technologies required precise identification of immature germ cells sub-populations, for subsequent application in the intra-cytoplasmic injec-

tions on ICSI technique [21]. The results from the current investigation indicate an increased content of degenerating spermatids with picnotic nuclei and vacuolized cytoplasm, which are not appropriate for such purposes. The nuclear/chromatin defects that might occur in germ cells and their subpopulations as well as in mature spermatozoa demand careful choice in their using for ICSI procedures.

Additional studies are necessary in cases with *ascending testis* or similar pathologies (hyperthermia, cryptorhidism, varicocele), for a better clarification of the seminal fluid cytology and, hence, of the injuries in the human spermatogenesis.

Conclusion

The increased secretion of germ cells (spermatogonia, spermatocytes and spermatids) in the seminal fluid might be good indicators for the abnormal functions of the testis, and in this way, could provide information for the stage, in which the arrest in the germ cells development occurs. Further investigations are necessary due to limited information in the literature about the "round cells" presence in the seminal fluid, which also play a prognostic role for determination of the spermatogenesis defects and could be useful in the choice of appropriate therapeutic strategies in andrology.

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