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ACTUAL KNOWLEDGE AND FUTURE PERSPECTIVES ON OBSTRUCTIVE AZOOSPERMIA (META-ANALYSIS AND TEXTBOOK REVIEW)

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Abstract

In this paper are reviewed and analyzed parts of works of the most prominent scientists and clinicians in the field of male infertility recombined with studies conducted in the Republic of Macedonia. The material was collected from the databases of standard digital libraries.

Referring to the data that 10 million males out of 3 billion in a reproductive age are azoospermic, and a number that should not be neglected lives within the Balkan Peninsula, where documented ciphers are lacking on the prevalence of azoospermia.

Epigenetic changes are now known to affect gene expression and some genes participating in spermatogenesis have been demonstrated to be epigenetically regulated.

Laboratory management of gametes taken surgically requires special attention because sperm retrieval from azoospermatic males is often of compromised quality.

In the focus of study were the compared results of successful pregnancy rate, after intracytoplasmic sperm injection (ICSI) and the health of offspring originating from such fathers, with meta-analysis of predictive factors such as etiology, sperm injection technique, and clinical results from assisted reproductive technique (ART).

The development of artificial gametes presents challenge and biotechnological perspective. Stem cell research is the field of hope for treating the most severe cases of azoospermia and potential healing of male sterility.

Keywords: male infertility, obstructive azoospermia, perspectives, knowledge, ICSI, ART

Introduction

This review is fully dedicated to the topic of obstructive azoospermia and contains seminal work of the most illustrious scientist and clinicians from USA, Brazil, Europe and Asia. There are also reviewed some scientific papers done by Macedonian Academy of Sciences and Arts, section of Biological and Medical Sciences. Two major breakthroughs in the last three decades revolutionized the field of male infertility. The first was the development of intracytoplasmic sperm injection (ICSI) for the treatment of male factor infertility, and the second was application of ICSI in azoospermic males, with the demonstration that spermatozoa derived from either the epididymis or the testis, were capable of normal fertilization and pregnancy [1, 2].

Azoospermia, defined as a complete absence of spermatozoa in ejaculation, invariably results in infertility but does not necessarily implicate sterility [3]. Obstructive azoospermia (OA) has been attributed to a mechanical blockage that can occur anywhere along the reproductive tract, including the vas deferens, epididymis, and ejaculatory duct. OA is considered to be one of the most favourable prognostic conditions for male infertility because spermatogenesis is not disrupted, unlike in non-obstructive azoospermia (NOA) [3]. Furthermore, the correlation of increased risk for congenital anomalies and potential iatrogenic transmission of genetic disorders with ICSI using sperm retrieved from these patients is still under debate [4].

The treatment of Azoospermia has undergone a radical change over the past 17 years, evolving from a clinical diagnosis with no direct corrective options to a highly treatable entity. This was the result of ICSI, which was introduced in 1992, which eliminated many obstacles in the way of fatherhood for men with severe male factor infertility. Today, more than 95% of men can have their own genetic child without the need for sperm donation [5]. Treatment of azoospermia-related infertility now covers a wide interventional spectrum including genetic studies, hormonal control, microsurgical and medical therapy, assisted reproduction techniques (ART), and innovative stem cell research that aims to create artificial gametes. [6].

Aim of the study

The aim of this study is:

- To provide the researchers and those interested in this field with comprehensive review of the global epidemiology.
- To provide the latest information and innovations in genetics, physiopathology and diagnostic procedures.
- Meta-analysis of the predictive factors like etiology, sperm retrieving procedures and gamete source and whether this has an impact in ICSI results in OA men.
- To analyze pregnancy outcomes after ICSI and health condition of children born from fathers with OA.
- What are biotechnological perspectives and challenges in managing azoospermia and assessing the opportunities of researching in this field?

Material and methods

The information is obtained from the digital databases of the standard libraries like: EBSCO, PubMed, Medline, DynaMed and Europe PMC.

A systematic review of the literature was done, focused in studies that directly compared the pregnancy outcomes after ICSI and meta-analysis of the clinical results of ART preformed in men with OA.

Moreover, descriptively are represented achievements in the field of genetics, treatment, perspectives and challenges that male infertility field has to face out.

Results

Azoospermia is identified in approximately 1% of all men and 10% to 15% of infertile males [3]. With a population of approximately 3 billion people at reproductive age, a gross estimate indicates that approximately 10 million men worldwide are azoospermic [7]. Referring to this cipher, a considerable number of azoospermic males lives in the Balkan Peninsula, in which there is still no official data on the prevalence of this pathology, a scope which is not so well illuminated because of the traditional-classical thinking, that a male can never be the reason of not having an offspring.

Despite enormous progress in the understanding of human reproductive physiology, the underlying cause of male infertility remains undefined in about 50.0% of cases, which are referred to as idiopathic infertility [8]. Most idiopathic cases are likely to be of genetic origin because the number of genes involved in spermatogenesis is probably over 1 thousand [9]. Recently, the contribution of spermatozoa to the embryo has been better explained, with accumulative evidence suggesting that various spermatozoid components participate actively in early human development [10]. The role of spermatozoa as a highly specialized cell is not only to transmit the father's DNA to oocyte but also provides a powerful epigenetic contribution to embryogenesis transmitting the activating factor (important for fertilization) to the oocyte, centrosomes (important for cell division) and a host of informational RNAs and microRNAs. This complex complement of microRNAs and other non-coding RNAs is thought to be the key to post-fertilization modifying changes [11]. Genetic testing involving the Y chromosome can now identify accurately azoospermic males diagnosed with idiopathic infertility [12]. Moreover, Y chromosome testing is a prognostic value for sperm retrieval in NOA men [13].Y chromosome microdeletions represent the etiological factor of 10-15% of idiopathic azoospermia and severe oligozoospermia [14]. The frequency of AZF(azoospermia factor) deletions in infertile males varies from 5.0 to 20.0% in worldwide studies [15].

Regarding this field, there are several studies conducted by a group of authors belonging to the Research Centre for Genetic Engineering and Biotechnology from Macedonia. In their paper titled "AZF Deletions in infertile men from the Republic of Macedonia", the aim was to estimate the length and boundaries of AZF deletions and to

correlate the AZF deletions with the sperm concentrations, testicular histology, Y haplogroups and the ethnic origin of the men with deletions. Two hundred and eighteen infertile/subfertile males, attending the Andrology Outpatient Clinic, at the Endocrinology and Metabolic Disorders Clinic, Faculty of Medicine in Skopje, were enrolled in the study. Semen analysis showed azoospermia in 99 men. The screening was performed by two multiplex PCR reactions analyzing six STS loci in the three AZF regions (sY84 and sY 86 in AZFa; sY 127 and sY 134 in AZFb; and sY254 and sY 255 in AZFc). A total of nine men out of 218 infertile/subfertile men showed the presence of Y microdeletions. In eight patients the results were consistent with the presence of AZFc deletions, while in one patient a larger deletion involving both AZFb and AZFc regions was detected. Analysis of additional STSs showed that six patients had an identical deletion with proximal end within a 349 kb region bounded by STSs sY1192 and sY1197, and distal end within a 229 kb region bounded by STSs sY1054 and sY1125. Assuming the homologous recombination between amplicons b2 and b4, the size of this deletion was 3.5Mb. Individuals with the 3.5Mb AZFc deletion were of different ethnic origin, four were Macedonian, one was Albanian and one was Romany [16].

For a better diagnose Dr. Aziz stresses that proper techniques are needed to reduce the amount of analytical error and enhance sperm count precision when evaluating semen specimens. The correct assessment of an initially azoospermic semen specimen should be followed by an examination of the pelleted semen to exclude cryptozoospermia. He provides insightful information on the seminal plasma biomarkers that may aid in determining the causes of azoospermia, useful in discriminating NOA cases from OA [17].

Ejaculates of men with spermatogenic failure usually have normal volume and pH, which indicates both functional seminal vesicles and patent ejaculatory ducts. The lower reference limits for ejaculate volume and pH are 1.5 ml (5th percentile, 95% confidence interval 1.4-1.7) and 7.2, respectively [18]. Retrograde ejaculation should be suspected when ejaculate volume is <1 ml, which can be confirmed by the finding of spermatozoa in the post-ejaculatory urine [19].

Sperm retrieval techniques are surgical methods that were developed to obtain spermatozoa from the epididymis and testes of azoospermic men seeking paternity. Methods of surgical sperm retrieval include:

- PESA (Percutaneous Epididymal Sperm Aspiration),
- MESA (Microsurgical Epididymal Sperm Aspiration),
- TESA (Testicular Sperm Aspiration). This includes TFNA (testicular fine needle aspiration) and TESE (Testicular Sperm Extraction).

- **The impact of etiology on retrieval success and reproductive outcomes after ICSI**

Drs. Baker and Sabanegh from the Cleveland Clinic discuss the current indications, techniques and results of reconstructive procedures in OA. The authors indicate that the use of optical magnification is now the gold standard for vasal reconstruction [20].

In a study by Sukcharoen et al. comprising 17 patients and 21 ICSI cycles within a period of two years, subjects were divided into three groups according to the time elapsed since vasectomy: 0-10 years, 11-20 years, or more than 20 years. No difference could be found regarding fertilization rates (FRs), implantation rates (IRs), and pregnancy rates (PRs) between the groups [21].

In an earlier study by Chen et al., 31 patients with obstructive azoospermia underwent epididymal aspiration procedures associated with ART (including IVF, zygote intra-Fallopian transfer, sub-zonal insemination). The fertilization rate for caput spermatozoa was lower than that for spermatozoa derived from other areas of the epididymis (p<0.05) [22].

In a meta-analysis involving 756 ICSI cycles using surgically retrieved sperm, Nicopoullos et al. compared the outcomes of ICSI between patients with congenital and acquired OA. The meta-analysis revealed no difference in either clinical pregnancy rate (relative risk [RR]: 1.03; 95% confidence interval [CI]: 0.75-1.31; p=0.87) or live birth rate (RR: 1.03; 95% CI: 0.81-1.31; p=0.80) between patients with congenital and acquired cases of OA.

A significantly higher fertilization rate was noted in the acquired group (RR: 0.92; 95% CI: 0.84-1; p=0.05), while a significantly higher miscarriage rate (MR) was noted in the congenital group (RR: 2.67) [23].

A more recent and larger series conducted by Kamal et al. involved 1,661 ICSI cycles in 1,121 men with proven histological OA. The outcomes were compared according to the sperm retrieval source and cause of obstruction. The fertilization rates (68.0% versus 64.2%, p=0.02), implantation rates (19.9% versus 20.8%, p=0.41), and frequencies of clinical pregnancies (43.2% versus 42.3%, p=0.84) and miscarriages (18.4% vs. 17.6%, p=1.0) resulting from the use of testicular and epididymal spermatozoa, respectively, were comparable in ICSI. Similar rates were maintained after stratification according to the cause of obstruction (Congenital Bilateral Absence of Vas Deferens (CBAVD) versus acquired obstruction), suggesting that neither the origin of surgically retrieved spermatozoa nor the cause of obstruction have any significant effect on the success of IVF/ICSI [24].

- **Impact of the method of collection on retrieval success and reproductive outcomes after ICSI**

Lin et al. analyzed 100 men with irreparable OA who underwent 109 ICSI cycles. The PESA SRR was 61%. MESA or testicular sperm extraction (TESE) were successfully performed if PESA failed. Fertilization and pregnancy rates were not significantly different for PESA-ICSI cycles (56 and 39%, respectively) and MESA-ICSI cycles (47 and 45%, respectively) [25].

PESA has been associated with better recovery of motile spermatozoa compared with testicular retrieval (TESA) (100% versus 39.3%, $p<0.0001$) in patients with azoospermia or severe oligozoospermia [26].

- **Impact of gamete source (epididymis or testicle) on reproductive outcomes with ICSI**

In early ICSI series, the fertilization and pregnancy rates using surgically retrieved spermatozoa obtained from the epididymis or testis were comparable to the results of ejaculated sperm [27,28].

In a meta-analysis by Nicopoullos et al., fertilization rates were reported to vary from 45 to 72% for epididymal and 34 to 81% for testicular sperm. Relative risk (RR) ratios of 1.08, 1.01, and 0.71 were described for the fertilization rate, clinical pregnancy rate and live birth rate, respectively, for epididymal compared with testicular sperm ($p>0.05$) [23]. Similar results were reported by Kamal et al.

Conversely, Dozortsev et al. reported higher FRs using spermatozoa retrieved from the epididymis compared with the testis in OA cases (77.2 versus 67.5%, $p=0.0005$). However, patients in the testicular sperm group exhibited significantly higher IR (20.8 versus 32.8%, $p=0.008$), with a trend toward higher ongoing PR and lower miscarriage rate. These authors speculate that motile sperm randomly taken from the epididymis have lower reproductive potential than random sperm taken from the testicle, and argue that the prolonged presence of sperm within the epididymis may lead to structural chromosomal aberrations that can compromise the reproductive potential of such cells [29].

- **Assessment of children born with ICSI using sperm retrieved from men with OA**

In a recent Dutch prospective, multicentre study by Woldringh et al., 378 children born from ICSI cycles using retrieved epididymal sperm were evaluated. More than 1,000 children born as a result of ICSI or IVF using ejaculated sperm were available for comparison. Assessments were performed at birth and at 1 and 4 years of age using mailed questionnaires. Moreover, follow-up visits of 2-year-old children were carried out to evaluate motor performance and mental-language development. The epididymal sperm group did not exhibit a higher incidence of stillbirths, malformations or poor development compared with the reference group of children born after ICSI or IVF using ejaculated sperm [30].

Discussion

Despite the advances in the diagnostic tools, many men are still classified as having idiopathic azoospermia[7]. In Macedonia there are only three private and one state clinic that treats cases of azoospermia and other forms of infertility, with a limited range of interventions. But the number of those seeking treatment is thought to be lower than it really is. Therefore, this is one of the reasons that we still do not have a clear cipher for men with azoospermia particularly and infertility generally.

The role of spermatozoa as a highly specialized cells with the purpose of not only delivering competent paternal DNA to the oocyte but also providing a robust epigenetic contribution to embryogenesis [9]. The paternal epigenetic contribution to embryogenesis requires that both the sperm DNA and the chromatin structure as a whole contain layers of regulatory elements that are sufficient to drive genes towards activation or silencing upon delivery to the egg [11, 12].

An accurate assessment of very low sperm counts aims to avoid labeling severely oligozoospermic men as azoospermic, which is particularly important in the current era of ART [17]. Currently, ART is the only option for most men with azoospermia-related infertility to have their biological offspring. Success has been achieved with ICSI in OA and NOA, and the use of non-ejaculated sperm coupled with ICSI has become a worldwide established procedure.

The laboratory management of surgically retrieved gametes requires special attention because spermatozoa collected from azoospermic men are often compromised in quality and more fragile [31]. Drs. Popal and Nagy from Atlanta provide strategies for handling such gametes inside the laboratory and discuss potential dangers [32].

The application of advanced laboratory technology and quality control are recommended to avoid jeopardizing the fertilizing potential of the sperm and chances of achieving a successful conception. The correct assessment of an initially azoospermic semen specimen should be followed by an examination of the pelleted semen to exclude cryptozoospermia, which is defined by the presence of a very small number of live sperm in a centrifuged pellet [20].

Despite being highly successful, microsurgical reconstruction may not be indicated in all men with OA, such as CBAVD patients and certain cases of post-infectious obstructions or failed vasectomy reversals [3]. The authors of the aforementioned study concluded that the interval between vasectomy and surgical sperm retrieval associated with ICSI treatment had no impact on pregnancy outcomes, although it should be noted that the analyzed sample was small and likely lacked statistical power to enable definitive conclusions [21]. The CBAVD group exhibited a higher probability to achieve pregnancy compared with the acquired obstruction group (20 versus 5.9%, $p>0.05$). Currently, ICSI is used rather than conventional IVF after sperm retrieval because sperm injection has been shown to result in a significantly higher fertilization rate [33].Spermatogenesis in CBAVD appears normal, but sperm derived from the caput epididymis are thought to have a low fertilizing capacity in conventional IVF cycles because of their short passage through the epididymis [34].

As for the ICSI cycles, for men with OA it was concluded that, the cause of OA appears to influence the outcome, with higher FRs and lower MRs observed in patients with acquired OA. However, tests of heterogeneity were significant, and it should be noted that the studies included had no power to detect clinically significant differences in the analysed outcomes [23].

Factors associated with the preference for one technique over the other include the quantity of retrieved spermatozoa and the ability to cryopreserve the excess retrieved sperm. [26].

In the studies by Nicopoullos et al. and Kamal et al., authors speculate that motile sperm randomly taken from the epididymis have lower reproductive potential than random sperm taken from the testicle, and argue that the prolonged presence of sperm within the epididymis may lead to structural chromosomal aberrations that can compromise the reproductive potential of the such cells [35].

To corroborate these findings, recent studies have demonstrated that epididymal sperm exhibit more DNA damage than sperm retrieved directly from the testis [35,36]. In the study conducted by Dozortsev et al. it was reported higher FRs using spermatozoa retrieved from the epididymis compared with the testis in OA cases. However, patients in the testicular sperm group exhibited significantly higher IR, with a trend toward higher ongoing PR and lower miscarriage rate [29].

As for the assessment of children born with ICSI using sperm retrieved from men with OA, no major difference has been reported in the short-term neonatal outcomes of children born from fathers with OA[30].

- **Development of artificial gametes**

Research toward the development of artificial gametes is timely due to the prevalence of Azoospermia and inability of harvesting mature sperm from the testes in approximately half of patients. In addition, the overall efficiency of spermatid injection is disappointing, and the reproductive potential after ICSI using testicular sperm retrieved from azoospermic men with dysfunctional spermatogenesis is only fair. A recent breakthrough report by Japanese scientists at Kyoto University used stem cells from mouse embryos to create primordial germ cells, which were then able to differentiate in spermatozoa after testis transplantation in mice [37]. Dr. Franca from the Federal University of Minas Gerais, Brazil, in collaboration with Dr. Schlatt from Munster, Germany are exploring the novel biotechnological methods to rescue fertility while maintaining biological fatherhood. Human haploid-like cells have already been obtained from pluripotent stem cells of somatic origin using the novel technique of in vitro sperm derivation [38]. Germ cell transplantation as a form of grafting is a promising method that may restore the fertility of pre pubertal boys who previously received cancer treatments [39]. Although promising, these methodologies are experimental, and the production of human gametes in the laboratory is a highly complex process that has yet to be translated to reproductive medicine [37].

This textbook review is the first of its kind in terms of pursuing innovations in the field of infertility in Macedonia. We hope to contribute generously to current scientific knowledge that includes azoospermia and its role in male reproductive health, as well as in developing a national strategy for the identification, classification and creating confidential databases.

Conclusions

1. Many men are still classified as having idiopathic azoospermia because the specific etiological factor remains unidentifiable. Therefore, determining the etiology of azoospermia remains one of the main challenges in this field.
2. In cooperation with infertility centres, it is necessary to build a more accurate database of cases with infertility and azoospermia and to see clearly the prevalence of male infertility in Macedonia.
3. Epigenetic changes are now known to affect gene expression and some genes participating in spermatogenesis have been demonstrated to be epigenetically regulated.
4. Proper techniques are needed to reduce the amount of analytical error and enhance sperm count precision when evaluating semen specimens.
5. No impact on pregnancy outcomes between vasectomy and surgical sperm retrieval associated with ICSI.
6. The cause of OA appears to influence the outcome, with higher FRs and lower MRs observed in patients with acquired OA.
7. Neither the origin of surgically retrieved spermatozoa nor the cause of obstruction have any significant effect on the success of IVF/ICSI.
8. When a diagnosis of OA is made, epididymal aspiration should be the retrieval method of choice in view of the possible complications of testicular extraction, including inflammation, hematoma, devascularisation and decreased serum testosterone levels.
9. ICSI performed with epididymal sperm does not lead to a higher rate of stillbirths or congenital malformations compared with IVF and ICSI with ejaculated sperm and does not lead to poor childhood development.
10. The development of artificial gamete presents the challenges and perspectives of biotechnology and stem cell research for treating the most severe cases of azoospermia and potentially "cure" male sterility.

Recommendations

We recommend the content of this paper not only for students and researchers in medical, veterinary and biological sciences but also for general practitioners involved in the management of infertile couples, urologists, andrologists, gynecologists, embryologists and reproductive specialists interested in following the exponential growth in the knowledge of azoospermia and infertility.

Nomenclature

OA – Obstructive Azoospermia
NOA – Non Obstructive Azoospermia
CBAVD - Congenital Bilateral Absence of Vas Deferens
IVF – In Vitro Fertilization
ICSI - Intracytoplasmic Sperm Injection
ART – Assisted Reproductive Technology
DNA – Deoxyribonucleic Acid
RNA – Ribonucleic Acid
AZF – Azoospermia factor
PCR – Polymerase Chain Reaction
STS – Sequence-Tagged Sites
PESA - Percutaneous Epididymal Sperm Aspiration
MESA - Microsurgical Epididymal Sperm Aspiration
TESA - Testicular Sperm Aspiration
TFNA - Testicular Fine Needle Aspiration
TESE - Testicular Sperm Extraction
FRs - Fertilization rates
IRs - implantation rates
PRs - pregnancy rates

MR – Miscarriage rate
RR - relative risk
CI - confidence interval

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