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REVIEW ARTICLE

The role of men in recurrent miscarriage; a Narrative Review

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Abstract: **Background and Method:** Recurrent Pregnancy Loss (RPL) is defined as the occurrence of two or more consecutive miscarriages within the first 20 weeks of pregnancy. The key challenge remains to be what it is that has to be done with couples experiencing RPL. Infections, endocrine disturbances, a suboptimal uterine environment, advanced maternal age, and genetic influences are some of the factors which may increase the chances of RPL occurrence. Despite all medical and research efforts, approximately 40 percent of RPL cases are categorized as unexplained. This is while amongst all the various factors which may lead to such condition, fathers' genetic influences have often been ignored in the past. In fact, parental chromosomal anomalies, gene mutations such as the microdeletion of chromosome Y, and/or some polymorphism of HLA-G have shown to contribute and lead to miscarriage. **Result:** Furthermore, high levels of Reactive Oxygen Species (ROS) can cause DNA damage in spermatozoa. Meantime, sperm DNA damage has been closely linked with indicators such as fertilization, embryo quality, implantation, spontaneous abortion, congenital malformations, and childhood diseases. Sperm Chromatin Structure Assay (SCSA) is a test to measure DNA fragmentation. Moreover, the other SCSA parameter that needs to be considered is high DNA stainability (HDS). It is worth mentioning that HDS is associated with frequency of aneuploidy in spermatozoa. In addition, high HDS can be associated with an increased risk of early abortion in IVF and ICSI cycles. **Conclusion:** Additionally, increasing paternal age and varicocele can increase the risk of miscarriage. Advanced Paternal Age (APA) can also increase the relative risk of offspring neurocognitive defects. In saying that, the microsurgical varicocelectomy effectively increases the odds of natural pregnancy, the rate of high-quality embryos, and the success rate of in vitro fertilization.

Keywords: Male; Miscarriage; Reactive Oxygen

1. Introduction

In academic terms, RPL is a heterogeneous condition and is defined as the occurrence of two or more consecutive pregnancy losses before 20 weeks of pregnancy (1). Between 6.5% and 21% of all clinically diagnosed pregnancies result in spontaneous abortion. In fact, higher rates are reported in infertile couples (2). The factors which may increase the risk of pregnancy loss are infections, endocrine disturbances, a suboptimal uterine environment, advanced maternal age, and other primarily genetic-based factors (3). It is worth mentioning that approximately 50% of RPLs are caused by chromosomal abnormalities, particularly aneuploidy (4). In addition, about 40 percent of RPL cases are categorized as unexplained, despite the extensive work that is employed by medical experts and researchers (5). In reality, the role of couples has proven to be a challenging task in the search for answers and potential methods to tackle this condition. This is while amongst all the various factors which may lead to such condition, fathers' genetic influences have often been ignored in the past. In other academic studies, aneuploid sets of chromosomes were seen in 0.6% of sperm in normal ejaculates, 6% in severe oligospermia, and 14% in nonobstructive azoospermia (6).

Spermatozoa with abnormal morphology not only affects fertilization, but also may result in a higher percentage of abnormal embryos which are aborted early in gestation. This

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observation emphasizes that sperm DNA integrity plays an important role in embryogenesis. Furthermore, sperm DNA damage may lead to pre- and post-implantation losses, early pregnancy loss, and congenital malformations (7). Karyotype cannot detect small chromosomal deletions which might result in pregnancy loss. In which case, Array Comparative Genomic Hybridization is necessary for the detection of these small chromosomal cuts. Recent evidence also proposes that the epigenetic changes of the sperm such as altered chromatin packing, and telomeric shortening, can play an important role in the etiology of RPL. It is also worth noting that paternal aging can also influence on embryonic development (8).

During normal spermatogenesis, most nuclear histones are replaced by protamines 1 and 2, creating a highly compacted nucleus containing the two protamines in an approximate 1:1 ratio. An abnormal P1-to-P2 ratio is also associated with reduced sperm concentration, abnormal sperm morphology, increased sperm DNA fragmentation, and reduced fertilization and implantation rates (9, 10). Human spermatozoa are also vulnerable to free radical attack by reactive oxygen species, this process destabilizes the DNA structure resulting in DNA strand breaks (11). Although the egg cytoplasm has the ability to repair damaged DNA, this ability may vary between individual eggs and amongst women of different age (12). In this review article, the male partner's role in pregnancy loss is to be presented and discussed.

2. Methods and Discussion

2.1. Chromosomal Abnormalities

Parental chromosomal anomalies were seen in 4% and 0.7% of couples with miscarriage and those from the general population, respectively (13). The genetic evaluation usually consists only of the karyotypes, and the most common finding is missing or additional chromosomes (3). Chromosomal abnormalities detected by karyotypes include abnormal structures of the individual chromosomes with the correct number of chromosomes (23 pairs or 46 chromosomes) or an abnormal total chromosome number (additional or missing chromosomes. e.g. 45, X or 47, XXY) (14) . Structural chromosome abnormalities are reported in about 3-6% of spontaneous abortions (15). In the study of human bodies, trisomy 21 and Klinefelter's syndrome have been shown to be 20% and 40% paternal in origin, respectively (15).

Individuals with balanced translocations will have no clinical or physical findings upon which to make the diagnosis, rather they can only be detected through a karyotype. Balanced translocations have a role on future pregnancy outcomes as they may produce balanced or unbalanced gametes. Balanced translocations consist of reciprocal or Robertsonian translocations or inversions. They are associ-

ated with reduced pregnancy rates and increased abortion (3). The incidence likelihood of Robertsonian translocation is about 0.1% in the general population (16) while is reported as high as 8% in couples with RPL (17).

2.2. Sperm Factors

Sperm DNA Damage

The most important change that occurs in spermatid is the exchange of 85% of the histones with protamine protein. After replacement, the protamine proteins cause folding of the DNA into a series of toroids, which allow for a high level of DNA compaction. This replacement and compaction not only reduce the head size of the sperm to enhance their hydrodynamics but also decrease the probability of DNA damage. However, about 15% of DNA is bound to histones. It is peripherally located in the nucleus and is susceptible to various environmental insults, especially oxidative damage (18, 19). It is obvious that any changes in genetic material of sperm may affect the zygote (20). Sperm DNA integrity is an important requisite for the correct transmission of genetic material to the offspring, and its impairment increases the risk of abortion (21). Sperm DNA or chromatin damage can be due to unrepaired DNA breaks, abnormal or incomplete chromatin packaging during spermatogenesis, abortive apoptosis, and oxidative stress induced by releasing of Reactive Oxygen Species (ROS) (22). The oxidative stress occurs when there is an imbalance between ROS and antioxidants. A small amount of ROS is necessary for the physiological function of sperm, including capacitation, hyperactivation, and acrosomal reaction. However, high levels of ROS can cause DNA damage in spermatozoa. Moreover, Oxidative Stress (OS) is mainly caused by factors associated with lifestyle (23). Sperm DNA damage has been closely linked with indicators such as fertilization, embryo quality, implantation, spontaneous abortion, congenital malformations, and childhood diseases (21). The assessment of the sperm quality based on the World Health Organization (WHO) guidelines are poor predictors of reproductive outcomes (24, 25). Different assays have been developed to assess sperm DNA damage, which are more clinically informative and relevant. The most used techniques for the analysis of sperm DNA fragmentation have traditionally been TUNEL test, Sperm Chromatin Structure Assay (SCSA), and Sperm Chromatin Dispersion (SCD) test. The TUNEL assay detects both single- and double-stranded DNA breaks by labeling the free 3'-OH terminus with the large terminal deoxynucleotidyl transferase (TdTA) enzyme. The SCSA test determines the percentage of sperm stained with AO in a semen sample that fluoresces red (broken DNA) or green (intact DNA) following an acid denaturation step (26). This is the only sperm DNA fragmentation test that simultaneously measures both DNA strand breaks and chromatin structure (27). The SCD test is based on the

principle that DNA fragments of sperm cannot produce a "halo" of dispersed DNA rings after acidic denaturation and nucleoprotein removal. The number of sperm without DNA fragments is assessed by microscopy (26). A significant negative correlation between sperm DNA damage and embryo quality has been elucidated in vitro and in vivo (27). The high degree of DNA fragmentation may not necessarily affect fertilization rates, while may result in subsequent miscarriage (5). Carlini et al. investigated 112 men from RPL couples, 114 infertile men with 1 or more impaired semen parameters, and 114 fertile men with high-quality semen, by analyzing the SDF using TUNEL. They found that the DFI was higher in the RPL group than that in the fertile controls (18.8% ± 7.0% vs 12.8% ± 5.3%, P < 0.001), and similar to that in infertile patients (28). Two recent cohort studies found SDF, measured with sperm chromatin dispersion test, is significantly higher in couples with unexplained RPL (URPL) compared to a control group of fertile men (29, 30). Therefore, it seems reasonable to offer SDF (Spermatozoa DNA Fragmentation) testing to couples with otherwise URPL. Besides advanced paternal age, many environmental factors, such as cigarette smoking, obesity, exogenous heat, and exposure to toxins, have been associated with increased SDF (30). Others, in contrast, reported that there was no significant correlation between DNA fragmentation and RPL. Thus, they concluded that DFI was not an important cause and predictive factor for RPL. Gil-Villa et al. evaluated the DFI in a control group (18.5% ± 4.2%) and a RPL group (16.3% ± 4.0%) using the SCSA test and found no significant difference between 23 couples with history of RPL and 11 men with recent fertility (31). Bellver et al. found that there was no statistically significant difference in the DFI (using the SCD test method) between a group of 30 patients with RPL and the 30 controls, and Coughlan et al. came to the same conclusion using the SCD test in 16 RPL patients (32). Menezo et al. reported that the use of oral antioxidant therapy could reduce the sperm DFI, especially in the setting of oxidative DNA damage, and significantly improve sperm DNA quality (33).

Another factor that has a possible role in RPL with high sperm fragmentation is the repair mechanism of the oocyte on sperm DNA damage. Hamatani et al. reported that sperm DNA may be repaired by oocytes up to a threshold of female age ≤35 years, thereby maternal age is an important factor in miscarriage (34). A study by Evenson and Wixon (2006) indicated a trend towards increased spontaneous abortions when the DFI was > 30%. DFI score > 30% was associated with increased miscarriage rates and a higher rate of spontaneous abortion at 12 weeks of gestation (P < 0.01) (35). lowering the DFI values to below the threshold of 13.59% through lifestyle management or medication is expected to increase the success rate of pregnancy in patients with repeated abortions (36).

2.3. Sperm chromosome: structural abnormality

The other SCSA parameter that needs to be considered is high DNA stainability (HDS) which expresses the fraction of sperm with higher level of green fluorescence due to a lack of full exchange of histones for protamines. Flow cytometric sorting demonstrates that the sperm with high HDS has a more rounded morphology than normal sperm, and lacks DNA strand breaks (37, 38). It is believed to characterize the immature sperm in the sample (38). Some studies suggest that semen samples with a high HDS level may lead to early embryo loss; however, the results are somewhat conflicting (39-41). HDS >15% was correlated with an almost 5% increase in the risk of early miscarriage (42).

Lin et al. (40) found that a high HDS was associated with an increased risk of early abortion in ICSI cycles, but not in IVF. Wyrobek et al. (43) found that HDS is associated with frequency of aneuploidy in spermatozoa (44). This is not surprising as immature sperms have an increased rate of aneuploidy and other chromosomal abnormalities (44). A higher proportion of immature spermatozoa (45, 46) as well as aneuploid gametes (47) have been linked to an increased risk of miscarriage after ART. It can therefore be speculated that the increased risk of miscarriage seen in couples with HDS >15% might be due to high aneuploidy rate in spermatozoa. It can also be speculated why the impact of high HDS on the risk of early miscarriage was only seen in ICSI cycles. One plausible explanation might be the difference in the mode of fertilization. In IVF, due to the competition between spermatozoa, it is likely that mature spermatozoa have a higher chance of binding to the zona pellucida and penetrating the oocyte. In ICSI, on the other hand, when using samples with high HDS, there is an increased risk of immature spermatozoa being injected into the oocyte, and therefore those pregnancies are at a higher risk of miscarriage (44). In support of this hypothesis, Lathi and Milki (48) found that a higher proportion of early aborted embryos with aneuploidy was seen in ICSI pregnancies compared with those resulting from IVF.

2.4. Gene Mutation

HLA-G

Mothers are anticipated to produce antibodies and a cytotoxic T lymphocyte response to foreign paternal HLA or other antigens expressed by the fetal cells (49). HLA Class Ia (A, B and C) are not expressed by trophoblasts to maintain pregnancy. A special set of HLA Class (Ib: E, F and G) is expressed on the fetal cells, which are thought to have an inhibitory effect on the maternal immune system. The expressions of class Ib antigens are organ-specific and conditional (50, 51). Several polymorphisms are present in the 3 prime untranslated region (30UTR) of the HLA-G gene. The polymorphisms in this region may have impact on the level of HLA-G ex-

pression and on pregnancy outcome (51, 52). The 14bp insertion/deletion polymorphism affects the stability of HLA-G mRNA and thereby the expression of HLA-G (53, 54). Aldrich and colleagues found that the HLA-G_ 0104 or HLA-G 0105N carrier in either partner was associated with recurrent miscarriage. These polymorphisms contribute to recurrent miscarriage (55).

2.5. Microdeletion of the Y Chromosome

Three AZF regions on the long arm of Y chromosome are necessary for normal spermatogenesis. AZF deletions have a negative influence on the sperm quality that may contribute to RPL. Y chromosome microdeletion is known to cause spermatogenetic failure and male infertility (56, 57). Y chromosome microdeletions occur in at least three regions, called azoospermia factor (AZFa, AZFb, and AZFc) (58). Recurrent deletions in the three AZF regions have been described in detail (59) which may lead to varying degrees of spermatogenic failure. Y chromosome microdeletions are detected in approximately 7% of men with oligozoospermia (59). Dewan et al. (60) found that the Y chromosome microdeletions are associated with RPL in severely oligospermic or azoospermic men. In this population, the prevalence of Y-chromosome microdeletions is estimated to be 8–18% (24, 61). In saying that, couples with unexplained recurrent pregnancy loss need to be tested to determine the Y-chromosome microdeletions.

2.6. Paternal age

In recent years, the cultural shift in delaying marriage and formation of families has risen concerns in regards to the effects of age on fertility. Currently, the effects of advanced maternal age are better understood than that of a father. Advanced Paternal Age (APA) is associated with declines in sperm quality which has impact on the rate of pregnancy and incidence of pregnancy loss (62). There is currently no agreed figure as to when the risks of adverse reproductive outcomes are significantly increased for men. Various studies defined different thresholds for advanced paternal age, the threshold > 30 years, or > 45 years, or 50 years are proposed (63). The American College of Medical Genetics (ACMG) has defined APA as 40 years or older at the time of conception (64). In a systematic review and metaâĂŘanalysis of 90 studies, Johnson et al reported that increasing paternal age contributes to the decline in semen volume, total sperm count and motility, percentage of morphologically normal sperm, and increased DNA fragmentation rates (65). Several studies show that DNA fragmentation increases with male age (43, 66). Several studies demonstrated that APA may negatively impact ART outcomes. In mostly retrospective studies, APA was associated with poor embryo quality (67), reduction of fertilization and implantation rates (68), and reduction in pregnancy and live

birth rates (68, 69). For APA couples undergoing IVF with ICSI, the negative impact appears to be limited to men with oligospermia (67, 70).

The risk of miscarriage was much greater for couples composed of a woman > 35 years and a man > 40 years. An association between paternal age and fetal loss strengthens the idea that paternal age influences the health of offspring via mutations of paternal origin (62). APA also increases the relative risk of offspring developing conditions such as neurocognitive defects, some forms of cancers such as leukemia, and syndromes related to aneuploidies (63). The effect of advanced paternal age on Klinefelter syndrome is also controversial. The frequency of XY spermatozoa, which would cause a 47, XXY condition in offspring, is higher in older men than in those who are younger (71).

2.7. Varicocele

The incidence of varicocele in men differs between 10% and 20% (72). Varicocele can cause sperm DNA damage, and elevated ROS and apoptosis rate (73). Excess ROS can cause pathological impairment in sperm DNA (73). Seminal antioxidant capacity significantly decreased in men with varicocele (74). Excessive levels of DNA damage contribute to a decrease in several fertility indices, including embryo cleavage rate, implantation rate, pregnancy rate, and live birth rate (75).

The risk of early miscarriage after varicocelectomy is about 15%. It is very similar to that reported in the general population. It has been shown that varicocelectomy decreases ROS levels and increases the antioxidant capacity of seminal plasma from infertile men with varicocele (75, 76). The microsurgical varicocelectomy effectively increased the odds of natural pregnancy, the rate of high-quality embryos, and the success rate of in vitro fertilization (75,77). If spontaneous pregnancy is not achieved within twelve months' post-surgery, an alternative approach such as ART treatment should be considered (78).

3. Conclusion

In terms of recurrent miscarriage, male factors such as chromosomal abnormalities, gene mutation, sperm DNA damage, paternal age, and varicocele, need to be taken in account. In fact, it seems reasonable to offer SDF testing and karyotype to couples with URPL. Furthermore, DNA fragmentation index and HDS are useful parameters in choosing the best treatment option. It is also worth noting that paternal age is an important factor especially in those couples where the female is older than 35 years of age. Lastly, microsurgical varicocelectomy increases the odds of live birth rate after ART.

4. Appendix

4.1. Acknowledgements

4.2. Authors Contributions

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4.3. Funding Support

4.4. Conflict of Interest

The authors declare that there is no conflict of interest in the publication of this paper.

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