COMMENTARY

Double strand DNA breaks in sperm: the bad guy in the crowd

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Abstract

Purpose The main objective of this opinion paper was to bring to light and enhance our understanding of the amount of double-strand DNA breaks in sperm and whether there is a threshold of no return when considering repair by the oocyte/embryo.

Methods A brief review of literature related to the theories proposed for the appearance of double-strand breaks in human spermatozoa. Further commentary regarding their detection, how oocytes or embryos may deal with them, and what are the consequences if they are not repaired. Finally, a strategy for dealing with patients who have higher levels of double-strand DNA breaks in sperm is proposed by reviewing and presenting data using testicular extracted sperm.

Results We propose a theory that a threshold may exist in the oocyte that allows either complete or partial DNA repair of impaired sperm. The closer that an embryo is exposed to the threshold, the more the effect on the ensuing embryo will fail to reach various milestones, including blastocyst stage, implantation, pregnancy loss, an adverse delivery outcome, or offspring health. We also present a summary of the role that testicular sperm extraction may play in improving outcomes for couples in which the male has a high double-strand DNA break level in his sperm.

Conclusions Double-strand DNA breaks in sperm provide a greater stress on repair mechanisms and challenge the threshold of repair in oocytes. It is therefore imperative that we improve our understanding and diagnostic ability of sperm DNA, and in particular, how double-strand DNA breaks originate and how an oocyte or embryo is able to deal with them.

Keywords Double strand DNA breaks · Testicular sperm · DNA repair · Sperm DNA

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The sperm DNA story

It is now more than 40 years since Evenson et al. [1] published a landmark study showing a relationship between mammalian sperm chromatin heterogeneity and fertility in a number of mammalian species, including humans. Ensuing studies have focused on the relationship between sperm chromatin/DNA damage and reproductive outcomes. Many of these studies have shown that men with high levels of sperm chromatin/DNA damage in their ejaculated sperm have a reduced chance of conceiving [2–6] and a higher incidence of fathering a pregnancy that could lead to a miscarriage [7–9]. Concurrently, the DNA sperm integrity tests available have still failed to convince many that they have clinical utility [10–12].

Although the predictive value of sperm chromatin/DNA fragmentation tests continues to be controversial, some recently introduced tests appear to significantly increase their clinical utility. Particularly those tests that measure double-strand breaks (DSB) in sperm, including COMET tests at neutral pH, which use specific software that makes test results more accurate, precise, and reproducible. The main objective of this opinion paper is to highlight the existence of DSB in sperm and postulate how they may arise and what their significance could be on the success of an IVF cycle.

The sensitivity to detect DSB is of paramount importance because, although DSB in sperm DNA are less frequent than single-strand breaks (SSB), they are highly deleterious, leading to genetic instability and chromosomal rearrangements [13]. Loss of control of DSB repair has gained increasing relevance since DSB repair plays a central role in the development of many human diseases [14]. Regarding embryo development, some studies suggest that the zygote responds to DSB in sperm DNA through mechanisms that delay the replication of paternal DNA, ultimately leading to embryo arrest [15, 16]. In particular, Casanovas et al. [16] showed that in patients with high levels of DSB, cleavage delay as assessed by morphokinetics is present throughout preimplantation development.

DNA double-strand breaks are repaired by means of two main mechanisms: nonhomologous end joining (NHEJ) and homologous recombination (HR) [17–19]. Both mechanisms operate in all eukaryotic cells that have been examined, but the relative contribution of each mechanism varies. For example, most mammalian cells seem to favor nonhomologous end joining. DSB restoration in the zygote occurs using NHEJ and HR repair pathways. These pathways are not equally important during the cell cycle. The choice of which repair pathway depends on the developmental stage of the embryo and the cell cycle. NHEJ works throughout the cell cycle, while HR functions during the S/G2 stage. DSB repair is obtained by stopping replication, and these breaks are preferably restored using HR [20]. In general, utilization of the DSB repair pathways during spermatogenesis from spermatogonia to sperm cells see the use of HR-based pathways in spermatogonia and spermatocytes, and the classical NHEJ pathway in spermatogonia, spermatocytes, round spermatids, and sperm cells. The alternative end joining (aEJ) pathway is utilized during spermatogenesis from spermatogonia to sperm cells except for spermatocytes (reviewed by [19]). It is believed that at the zygotic stage, NHEJ plays an essential role in the restoration of sperm DSBs [21]. Although some initial research has indicated the ability of the mammalian oocyte/embryo to repair DSB in sperm, little is understood of the consequences and impact it may have on the ensuing embryo and fetus. In particular, enhancing our understanding of the amount of DSB in sperm and whether there is a threshold of no return when considering repair by the oocyte/embryo is an area where more research should be targeted.

Why should we fear DSB in comparison to SSB and how do they originate?

There are several mechanisms of generation of DSB at the intra-testicular level: (i) during mitosis in spermatogenesis, DNA repair systems fail to repair DSB [22], (ii) during meiosis-I, ATM kinase fails to phosphorylate H2A histone at nuclear foci of DSB, enabling their identification and repair [23], and (iii) during spermiogenesis, topoisomerases play a dual role of DNA nucleases and ligases in order to provide relief of the torsional stress produced during the displacement of histones by protamines. If these breaks are not repaired by the ligase activity of topoisomerases, this will result in the generation of DSB followed by irreversible DNA degradation by a nuclease [15, 24–26]. It has been postulated that an intricate partnership also exists between topoisomerase integrated in the sperm chromatin and SUMOulation [27]. It would be thought that DSBs related to meiosis and mitosis would be targeted for clearance by apoptosis or by the Sertoli cells [22, 28–31]; however, the possible escape of sperm carrying these abnormalities is highly feasible. DSBs generated by anomalies in the final stages of histone-protamine replacement during spermiogenesis would less likely be policed by clearance mechanisms and could find their way into the ejaculated sperm population.

The aim of spermatogenesis is to create a highly organized and condensed chromatin sperm nucleus. This leads to an almost inert chromatin and shuts down gene transcription. The intricate folding needed to create this highly inert chromatin involves specific regions involving protamine and DNA toroids, which are thought to be connected to

Table 1 A summary of selected studies comparing fertilization, embryo development, and ongoing or live birth outcomes in patients using ejaculated and testicular sperm in relation to previous DNA fragmentation measurements or comparison of similar groups where male factors with low sperm counts were treated with testicular sperm. All cases utilized ICSI. Studies in grey indicate when the same patients had transfers using ejaculated and testicular sperm. Numbers highlighted in bold indicate a significant difference reported in the study

Author	Ref.	Type of sperm sample	Number of embryo transfers	Etiology of patients studied	Sperm DNA fragmentation method (%)	MII (mean)	Fertilization Rate 2PN/MII (%)	Embryo cleavage rate (%)	Day 5 blastocysts per day 2 embryos (%)	Clinical pregnancy (%)	Implantation rate (%)	Miscarriage after clinical pregnancy (%)	Ongoing pregnancy Per transfer (%)	Live birth Per transfer (%)	No. of live born (%)
Sousa (N = 127)	[35]	Ejaculate	47	RIF	None	7.3	63.6	91.0	47.1	10.3	5.6	50.0	5.1	5.1	5.1
		Testicular	80			7.1	72.6	94.8	62.2	39.1	26.6	25.9	27.5	27.5	31.9
Kahraman (1996) (N = 24)	[53]	Ejaculate	10	AT	None	-	54.5	94.4		20.0		100.0	0.0	0.0	
		Testicular	14	AT		-	53.5	96.3		57.1	20.7	25.0	42.9		
Greco (2005) (N = 36)	[54]	Ejaculate	18	RIF + hSDF	TUNEL (23.6%)	10.3	70.8	94.7	-	5.6	1.8	5.6	0.0	0.0	0.0
		Testicular	18		TUNEL (4.8%)	10.4	74.9	95	-	44.4	20.7	0.0	44.4	44.4	66.7
Hauser (2011) (N = 93)	[55]	Ejaculate Crypto-zoospermia	34	Crypto-zoospermia	None	9.0	38.2	54.5		14.3	5.1		14.3	14.3	11.8
		Testicular	59			9.1	48.4	54.3		31	12.1		27.9	27.9	18.6
Ben-Ami (2013) (N = 116)	[56]	Ejaculate	68	Cryptoz + RIF	None	8.1	38	69.7		15.1	5.7	2.9		9.4	
		Testicular	48			10.2	46.7	78.4		42.5	20.7	12.5		27.5	
Arafa (2018) (N = 72)	[57]	Ejaculate	36	hSDF + RIF	SCD (56.4%)	-	46.58	97.7	-	13.5		5.6	13.5	8.3	8.3
		Testicular	36		SCD (15.3%)		47	95.6		38.9		0.0	38.0	36.1	47.2
Herrero (2019) (N = 145)	[58]	Ejaculate	68	RIF + RPL	TUNEL (27.3%) SCSA (20.9%)	6.5	63.6			10		41.7		11.4	
		Testicular	77		TUNEL (32.2%) SCSA (28.8%)	6.5	62.7			27.9		25.0		23.4	

For abbreviations and explanations, see Supplemental Table 1key

Fig. 1 Consequences of ferti-
lization by a sperm carrying
double-strand breaks (DSB)
in relation to the threshold of
repair by the oocyte during
fertilization. High levels of
remaining DNA damage in the
oocyte will lead to failure at
earlier stages of development
(fertilization or embryo), while
lower levels of remaining DNA
damage may manifest them-
selves later in development
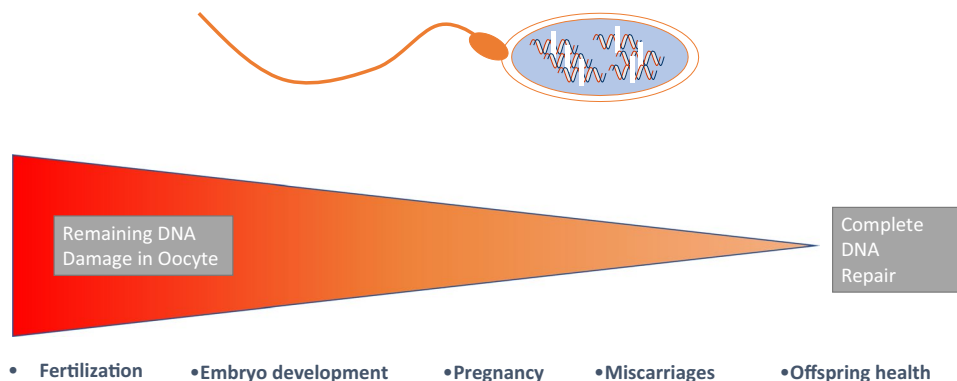
the nuclear matrix by toroid linker regions (see review by [32]). It has been postulated that these toroid regions in fully condensed sperm chromatin provide vulnerable, nuclease-sensitive regions that can be digested by external nucleases, leading to DNA strand breaks [32].

A further occurrence of DSB could manifest at the post-testicular level. We have proposed the occurrence of DSB during migration of spermatozoa through the epididymis via activation of endonucleases present in the nucleus of mature spermatozoa. Since oxygen radicals have been shown to activate sperm endonucleases [28], and it is well known that the levels of oxygen radicals in the epididymis can be relatively high [30, 33, 34], we postulate that DSB could also be generated during migra-tion of spermatozoa through the epididymis. In support of this hypothesis, we can examine whether there is a difference in sperm quality and reproductive outcomes between sperm that is recovered in the testes versus that recovered in the ejaculate.

Outcomes in patients treated with testicular sperm

Numerous publications have shown that males who have increased levels of DNA strand breaks in their ejaculated sperm show lower levels of DNA strand breaks in their sperm retrieved from the testes (Table 1, Supplemental Table 1). A group of studies have also shown improvement when reverting to the use of testicular sperm after poor outcomes when using ejaculated sperm (Table 1). These studies have in general been retrospective in nature, com-paring previous failed attempts with ejaculated sperm to those using testicular sperm (Table 1). The live birth out-comes in these selected patients are encouraging, and we would argue warrant a randomized trial to substantiate the growing data. The improvement in sperm characteristics is most convincing when examining the presence of DSB using various sperm DNA assessment techniques (Table 1, Supplemental Table 1). Furthermore, other parameters

show improvement when using testicular sperm, includ-ing an improvement in blastocyst development [35] and a decrease in miscarriage rates. One of the more intrigu-ing data sets supporting the induction of post-testicular DSB by activation of human sperm endonucleases in the epididymis is the improvement in pregnancy outcome observed in couples with repeated idiopathic IVF fail-ure and embryo cleavage arrest using ejaculated sperm when compared to the use of testicular sperm in TESE-ICSI cycles [36]. Overall, the drawback of the majority of studies published examining this question is that they are comparing previous failed cycles and making the associa-tion with high DNA strand break levels in the ejaculated sperm. The direct improvement when using testicular sperm, which possess lower levels of DNA strand breaks, must be verified in more robust studies.

Other approaches to limit DSBs in sperm prior to ICSI

Infertile males will routinely present with both SSB and DSB in their sperm population [7, 37]. The origin of these separate populations is speculative. While DSB may originate from earlier meiotic or chromatin reor-ganization, SSB have been largely linked with reactive oxygen species [38]. These reactive species also affect sperm motility by affecting the mitochondrial mem-brane. For this reason, any sperm selection system that selects progressively motile sperm (or eliminates immo-tile sperm) will be efficient in reducing single-stranded DNA-affected sperm [39]. On the contrary, DNA DSB could be present in spermatozoa with good motility and morphology. A number of other techniques have been proposed to eliminate sperm-carrying DSBs. In these cases, some microfluidic selection systems have proven to be effective in reducing these spermatozoa [40, 41]. Density gradient centrifugation has also been reported to have some efficiencies in removing sperm possessing DSB [42]. Unfortunately, the data examining the utility

of microfluidic systems is still minimal. Finally, the interest in sperm selection techniques is growing rapidly, as evidenced by a number of recent reviews [43–45] that have examined simple solutions such as short abstinence [46] to more complex artificial intelligence-based technologies [47].

How can DSBs in sperm be the bad guy?

We have known for many years that the oocyte and developing preimplantation embryo depend on a multitude of highly orchestrated and synchronized events to develop and reach their final goal of a healthy live birth. Sperm are implicated in many of the major early hurdles in development, including activation of maternal mRNA stores in the first few days of development, embryonic genome activation, metabolic switches, compaction, and differentiation of cell lineages in the blastocyst, to name a few of the critical events that must occur to ensure viability [48, 49]. Stress from any of these events can have long-term consequences. Fertilization from a sperm possessing either SSB or DSB will incur stress on the oocyte during fertilization and the developing embryo. We have already seen the human embryo is armed with a plasticity that can cope with adverse events [50]. The best-emerging example is that human blastocysts that have been clinically diagnosed as mosaic aneuploid (displaying an abnormal copy number of chromosomes) can lead to healthy births, suggesting the presence of an in vivo mechanism to eliminate aneuploidy [51, 52]. As controversial as this may be, it does indicate that mechanisms also exist in the oocyte or embryo to repair both SSB and delivered by a fertilizing sperm. We propose that a threshold may exist that allows either complete or partial DNA repair. The closer that an embryo is exposed to the threshold, or if it cannot maintain the threshold, then depending on the level, the ensuing embryo will fail to reach various milestones, including blastocyst stage, implantation, pregnancy loss, an adverse delivery outcome, or offspring health (Fig. 1). Sperm DSB would obviously provide greater stress on repair mechanisms and challenge the threshold more often. It is therefore imperative that we improve our understanding and diagnostic ability of sperm DNA, and in particular, how DSB originate and how an oocyte or embryo is able to deal with them.

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Declarations

Conflict of interest The authors declare no competing interests.

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