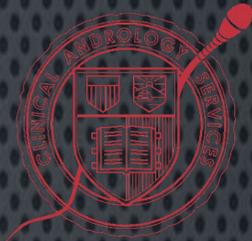




Choosing the Appropriate Insemination Method in Assisted Reproductive Treatments Based on Sperm Chromatin Fragmentation



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ABSTRACT

Study question: We question if sperm chromatin fragmentation (SCF) can guide couples with unexplained infertility and poor intrauterine insemination (IUI) outcomes to the appropriate subsequent reproductive treatments.

Summary answer: Couples with prior IUI failures can be offered a SCF-based treatment algorithm comprising of conventional IVF or ICSI with ejaculated or surgically retrieved spermatozoa. What is known already: Ovulation induction (OI) with IUI is the mainstay of treating couples with unexplained infertility. However, some couples may have poor IUI outcomes despite an adequate number of treatment attempts and normal semen parameters. Recent evidence has suggested that sperm DNA integrity is closely associated with fertility potential. During the later stages of spermiogenesis, breakage of a sizable amount of single- or double-stranded DNA occurs to allow tight chromatin compaction and, in ideal conditions, only those spermatozoa with fully repaired chromatin would reach the ejaculate. Thus, high SCF in the ejaculate, may have an inverse relationship with IUI success.

Study design, size, duration: Over a 39-month period, couples diagnosed with unexplained infertility, based on normal female infertility screening and semen analysis, underwent treatment with ovulation induction and IUI. Those with a history of 2-4 failed IUI attempts underwent SCF assessment of the ejaculate. The couples were then allocated to conventional IVF or ICSI with ejaculated or surgically retrieved sperm according to the level of SCF. The clinical pregnancy rate (CPR) was stratified according to the SCF-based insemination method.

Participants/materials, setting, methods: Infertile couples with unexplained infertility underwent SCF assessment by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay or sperm chromatin structure assay (SCSA). In-house TUNEL assessment evaluated at least 500 spermatozoa under fluorescent microscopy and the SCF was deemed abnormal above a threshold of >15%. SCSA assessment was also performed by an external laboratory, which analyzed at least 5000 spermatozoa. The SCSA was considered abnormal above a threshold of >25%.

Main results and the role of chance: A total of 645 couples underwent 1478 IUI cycles. The female age and male age in the study cohort was 37.7±4 years and 39.8±5 years, respectively. The overall average sperm concentration was 50.4±32 x10⁶/mL, with a motility of 46.6±12% and morphology of 3.1±2%. The CPR with IUI at our center in an age-matched cohort was 17.9%; however, the study cohort had CPR of 5.1%. The mean TUNEL and SCSA values of the men in the study cohort was 21.5 ± 8% and SCSA 39.5 ± 26%, respectively. Couples with failed IUI attempts but normal SCF in the male partner, underwent conventional IVF, resulting in a CPR of 23.8%. In women <35 years undergoing conventional IVF, the CPR increased to 38.3% (*P*<0.001). Couples with abnormal SCF underwent ICSI with ejaculated sperm, leading to an overall CPR of 24.1% (*P*<0.001); when controlling for female age <35 years, the CPR rose to 27.8%. Couples who failed to conceive with ICSI using ejaculated sperm were offered testicular sampling. Eighty-six men consented for the surgical retrieval were found to have a SCF on testicular sperm of 9.28± 6%, which was significantly lower than the SCF of their ejaculates (*P* < 0.001). The CPR in couple undergoing ICSI with surgically retrieved sperm was 33.0% and in women <35 years was 42.1%.

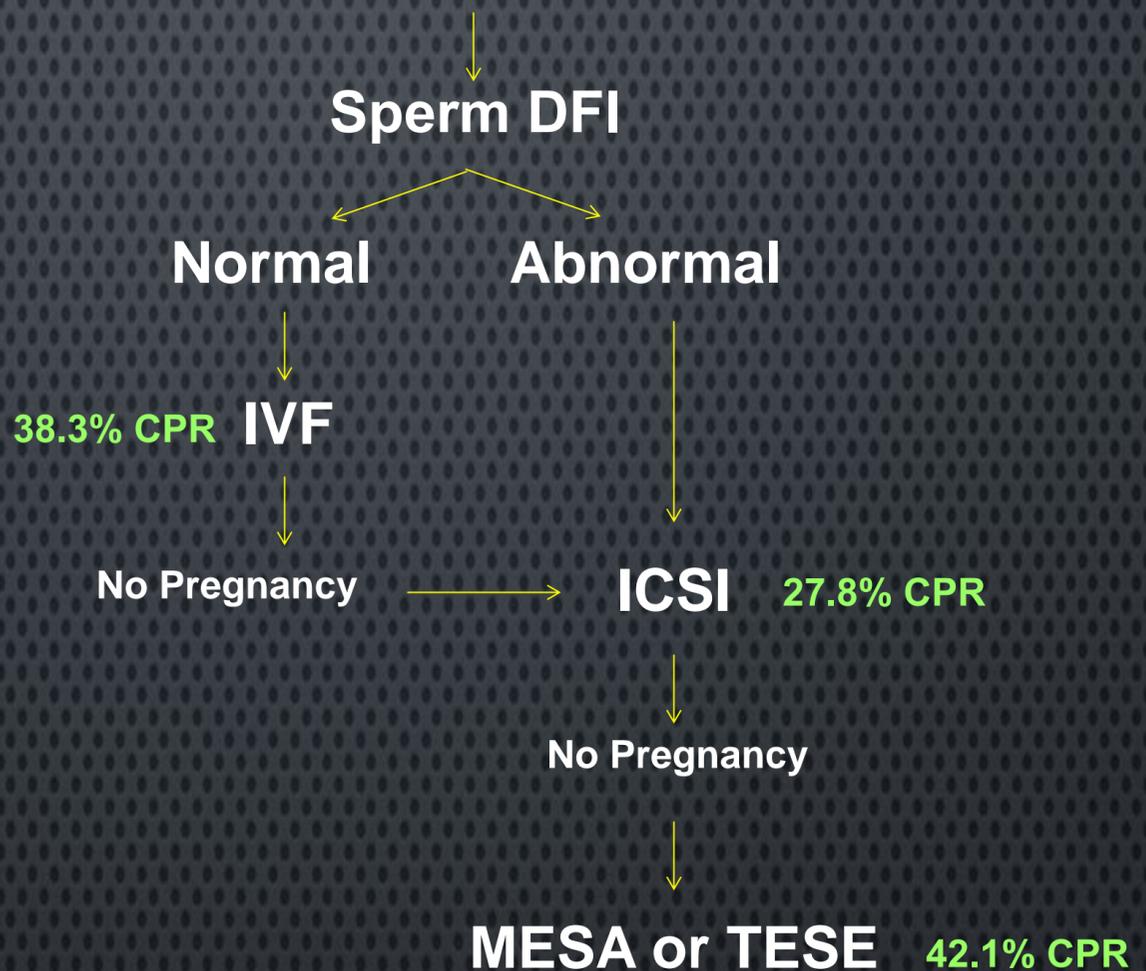
Limitations, reasons for caution: Surgical sampling of the vas deferens, epididymis, or testis in men with high SCF in their ejaculates samples or those with poor IUI outcomes should only be performed after extensive individualized counseling. Moreover, such an approach is preliminary and requires further prospective analysis.

Wider implications of the findings: IVF is successful in men with intact sperm chromatin. When sperm SCF is compromised in the ejaculate, ICSI is the most suitable insemination method. In men with high SCF in their ejaculate who fail ICSI treatment, surgical sampling yields spermatozoa with lower SCF and higher changes of pregnancy.

RESULTS

The CPR with IUI at our center in an age-matched cohort was 17.9%; however, the study cohort had CPR of 5.1%. The mean TUNEL and SCSA values of the men in the study cohort was 21.5 ± 8% and SCSA 39.5 ± 26%, respectively. Couples with failed IUI attempts but normal SCF in the male partner, underwent conventional IVF, resulting in a CPR of 23.8%.

IUI Poor Pregnancy Outcome 5.1% CPR



BACKGROUND

During the late stages of spermatogenesis DNA breakages are physiologically induced to allow tight chromatin compaction and only those spermatozoa with repaired chromatin reach the ejaculate. Throughout the male genital tract oxygen-free radicals, mostly from decaying spermatozoa and other cells, are the main cause of DNA damage and responsible for the impaired ART outcome. Therefore, in men with adequate semen parameters but poor reproductive outcome, a SCF assessment may guide towards the most effective ART treatment.

METHODS

A total of 645 couples with unexplained infertility underwent sperm chromatin fragmentation (SCF) assessment by TUNEL assay or SCSA. In-house TUNEL assessment evaluated at least 500 spermatozoa under fluorescent microscopy and the SCF was deemed abnormal above a threshold of >15%. SCSA assessment was also performed by an external laboratory, which analyzed at least 5000 spermatozoa. The SCSA was considered abnormal above a threshold of >25%.

Table.1 Parameters of semen analysis of men according to WHO 2010 criteria

No. of	
Couples	645
Female age (M yr ± SD)	37.7 ± 4
Male age (M yr ± SD)	39.8 ± 5
Concentration (Mx10 ⁶ /mL ± SD)	50.4 ± 32
Motility (M% ± SD)	46.6 ± 12
Morphology (M% ± SD)	3.1± 2

CONCLUSIONS

In patients with unexplained poor IUI outcome, SCF assay is helpful in devising treatment algorithm. IVF is successful in men with intact sperm chromatin. When SCF is compromised in the ejaculate, ICSI is the most suitable insemination method. In men with high SCF in their ejaculate who fail ICSI treatment, surgical sampling yields spermatozoa with lower SCF and higher changes of pregnancy.

Figure 1. Clinical Pregnancy Rate (CPR) of each insemination method with respect to normal and abnormal DNA fragmentation.

In women <35 years undergoing conventional IVF, the CPR increased to 38.3% (*P*<0.001). Couples with abnormal SCF underwent ICSI with ejaculated sperm, leading to an overall CPR of 24.1% (*P*<0.001); when controlling for female age <35 years, the CPR rose to 27.8%. Couples who failed to conceive with ICSI using ejaculated sperm were offered testicular sampling. Eighty-six men consented for the surgical retrieval were found to have a SCF on testicular sperm of 9.28± 6%, which was significantly lower than the SCF of their ejaculates (*P* < 0.001). The CPR in couple undergoing ICSI with surgically retrieved sperm was 33.0% and in women <35 years was 42.1%.

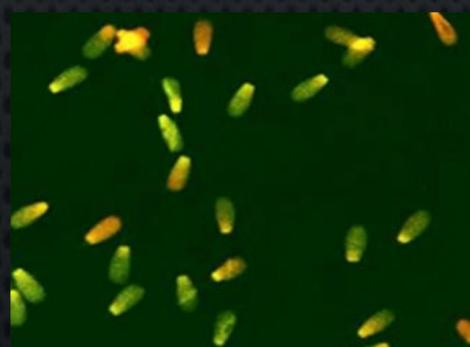


Figure 2. Sperm cells stained with acridine orange. Sperm cells with little or no detectable DNA damage will have a green light whereas sperm cells with moderate to severe DNA damage will have a yellow or red fluorescence respectively.

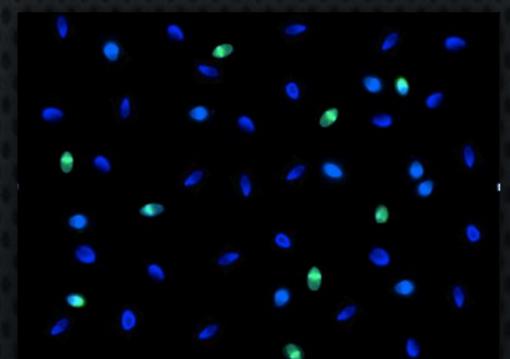


Figure 3. Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling (TUNEL) with DAPI counterstain (in blue). Green fluorescence indicates spermatozoa with SCF.