The value of semen analysis in assessment of male fertility past, present and future

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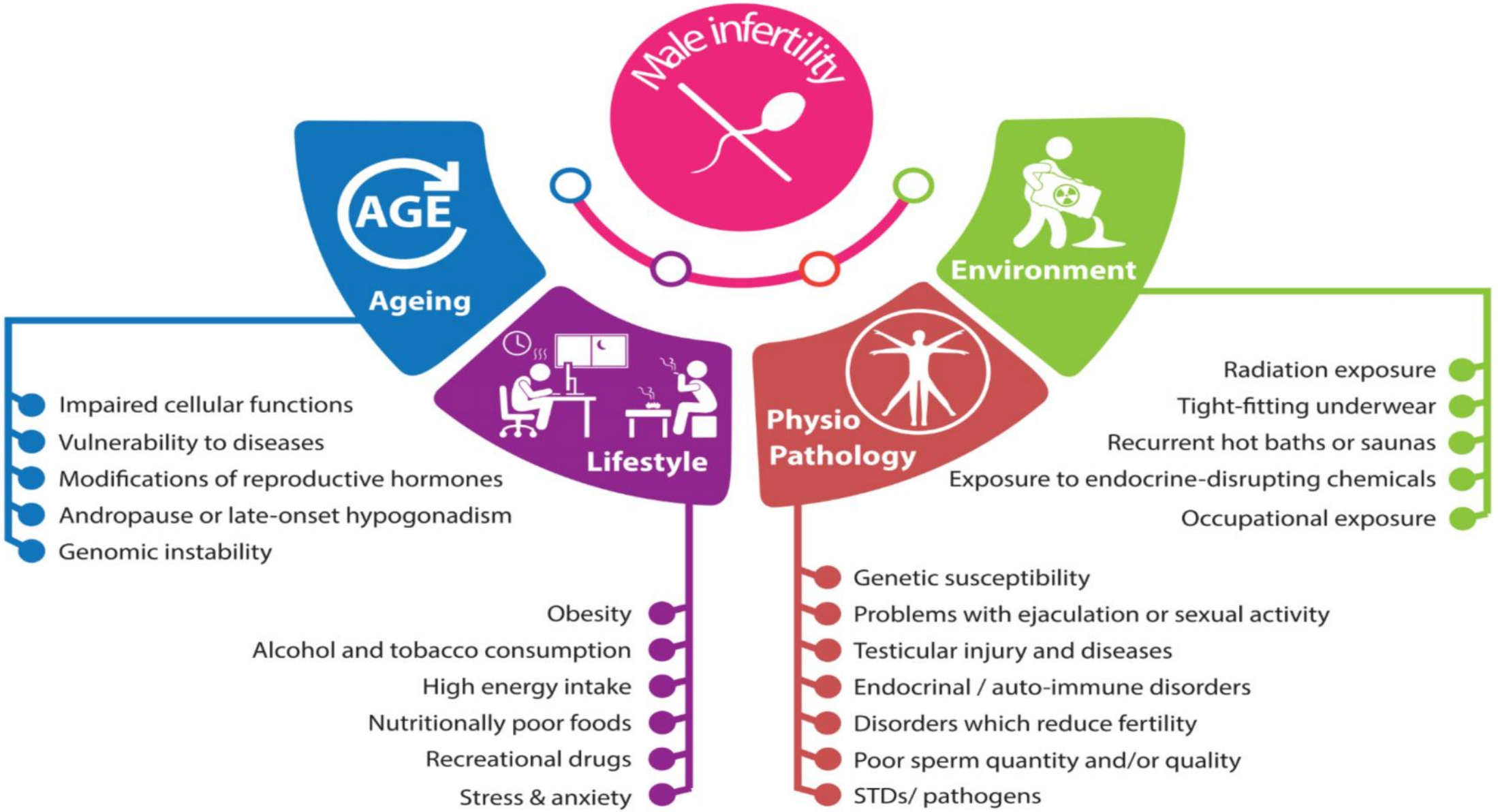
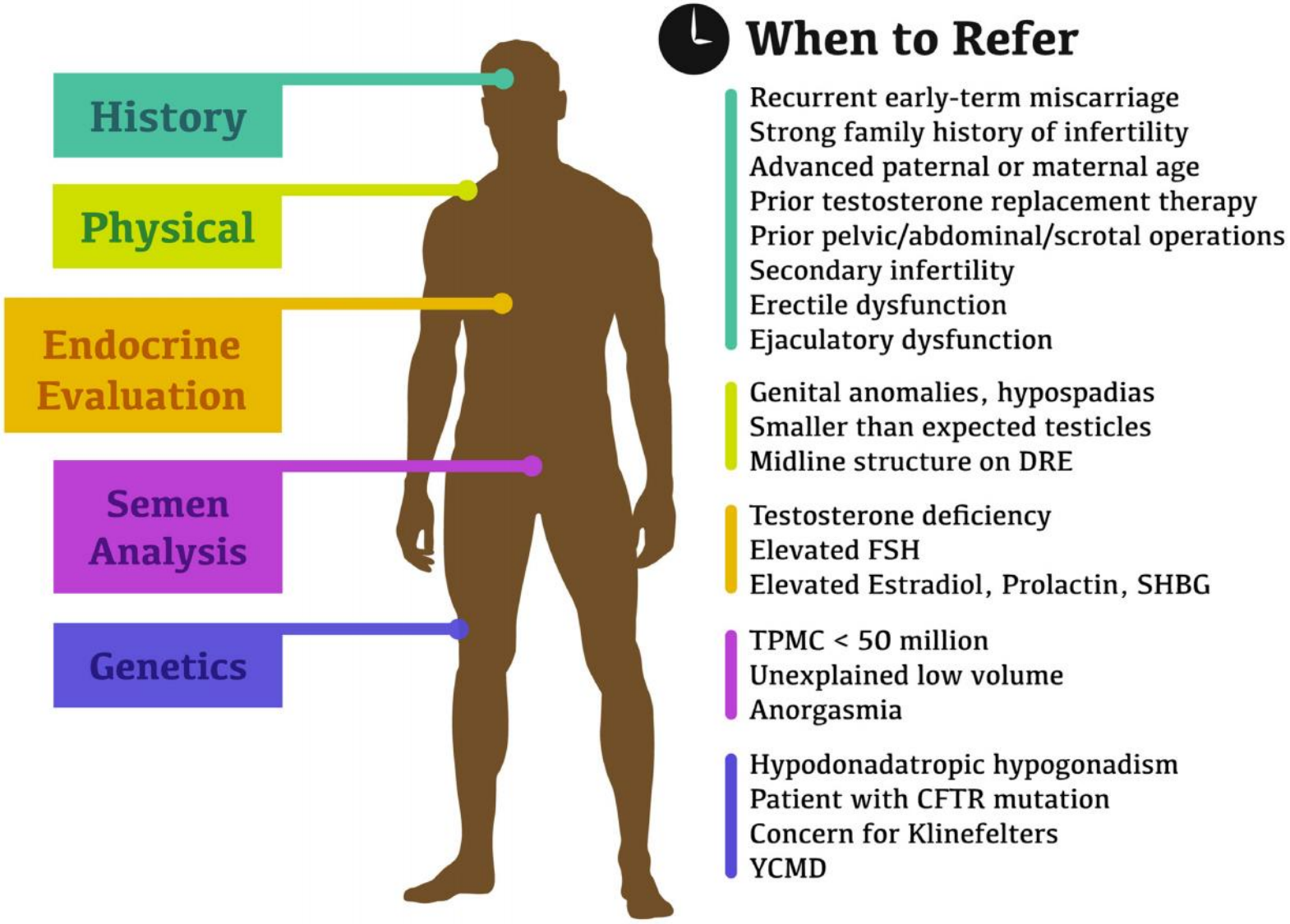

Male infertility
AGE
Ageing
Impaired cellular functions
Vulnerability to diseases
Modifications of reproductive hormones
Andropause or late-onset hypogonadism
Genomic instability
Lifestyle
Obesity
Alcohol and tobacco consumption
High energy intake
Nutritionally poor foods
Recreational drugs
Stress & anxiety
Physio Pathology
Genetic susceptibility
Problems with ejaculation or sexual activity
Testicular injury and diseases
Endocrinal / auto-immune disorders
Disorders which reduce fertility
Poor sperm quantity and/or quality
STDs/ pathogens
Environment
Radiation exposure
Tight-fitting underwear
Recurrent hot baths or saunas
Exposure to endocrine-disrupting chemicals
Occupational exposure



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Male Fertility Assessment

- Semen Analysis
- Endocrine test
- Genetic evaluation
- Imaging
- Testicular biopsy
- Sperm function tests

Semen analysis parameters, adjunct testing, and interpretation

Parameter	Ref (5^{th})	Clinical implications	Key studies
Volume	>1.4 mL	•Ejaculatory duct obstruction (EDO): •Retrograde ejaculation: postejaculatory urinalysis, prior pelvic surgery, medication review •Failure of emission: historical trauma, retroperitoneal or pelvic surgery, medication review •Agenesis/aplasia of SV's or prostate:	-2/3 of ejaculate volume comes from the SV, 1/3 from the Prostate, and <10% from the testicles and other accessory structures -Ejaculation volume increases by 11.9% per day during the first 4 d of the abstinence period. -Surgical treatment in infertile men with EDO may increase ejaculate volume up to 38–79% .

pH	≥7.2	•Seminal vesicles produce alkaline coagulum, and the prostate contributes acidic fluid. pH < 7.2 may indicate an obstruction or absence of seminal vesicles. Low-volume acidic azoospermia suggests bilateral EDO or congenital bilateral absence of the vas deferens. •pH of 8 may indicate infections •Alkaline pH could be because of poor sample handling and delay in analysis	•Reduced Na/K-ATPase activity at acidic pH's result in decreased sperm movement and capacitation. •Semen volume and total motility are positively correlated with pH .

Concentration	≥15 x10^6 /mL	•Oligozoospermia (< 15 x 10^6/mL): Endocrine evaluation •Severe oligozoospermia (<5 x 10^6/ml) or nonobstructive azoospermia: endocrine evaluation, karyotype testing, assessment of Y chromosome microdeletions •Incomplete congenital obstruction of the genital tract suspected: Cystic fibrosis evaluation, abnormal Mullerian structure development.	•Sperm concentration between subfertility range (13.5×10^6/ ml) and fertility range (48.0×10^6/ml) are associated with indeterminate fertility.. •YCMD are more prevalent in men with azoospermia, and are rarely seen in men with sperm concentration of 2–5 × 10^6/mL.

Total Count	≥39 x10^6	•All patients with nonobstructive azoospermia owing to primary testicular failure should be offered genetic testing. •Imaging for renal abnormalities in unilateral vasal agenesis or CBAVD with no evidence of CF.	•Genetic causes of male infertility are seen in 2.5% of patients with total sperm number between 1 and 10 × 10^6/ejaculate and in 10.8% of men with <1 × 10^6/ejaculate. ••TESE/ICSI could be helpful in majority of patients with obstructive azoospermia. ••Microdissection is safer than the conventional technique in nonobstructive azoospermia.

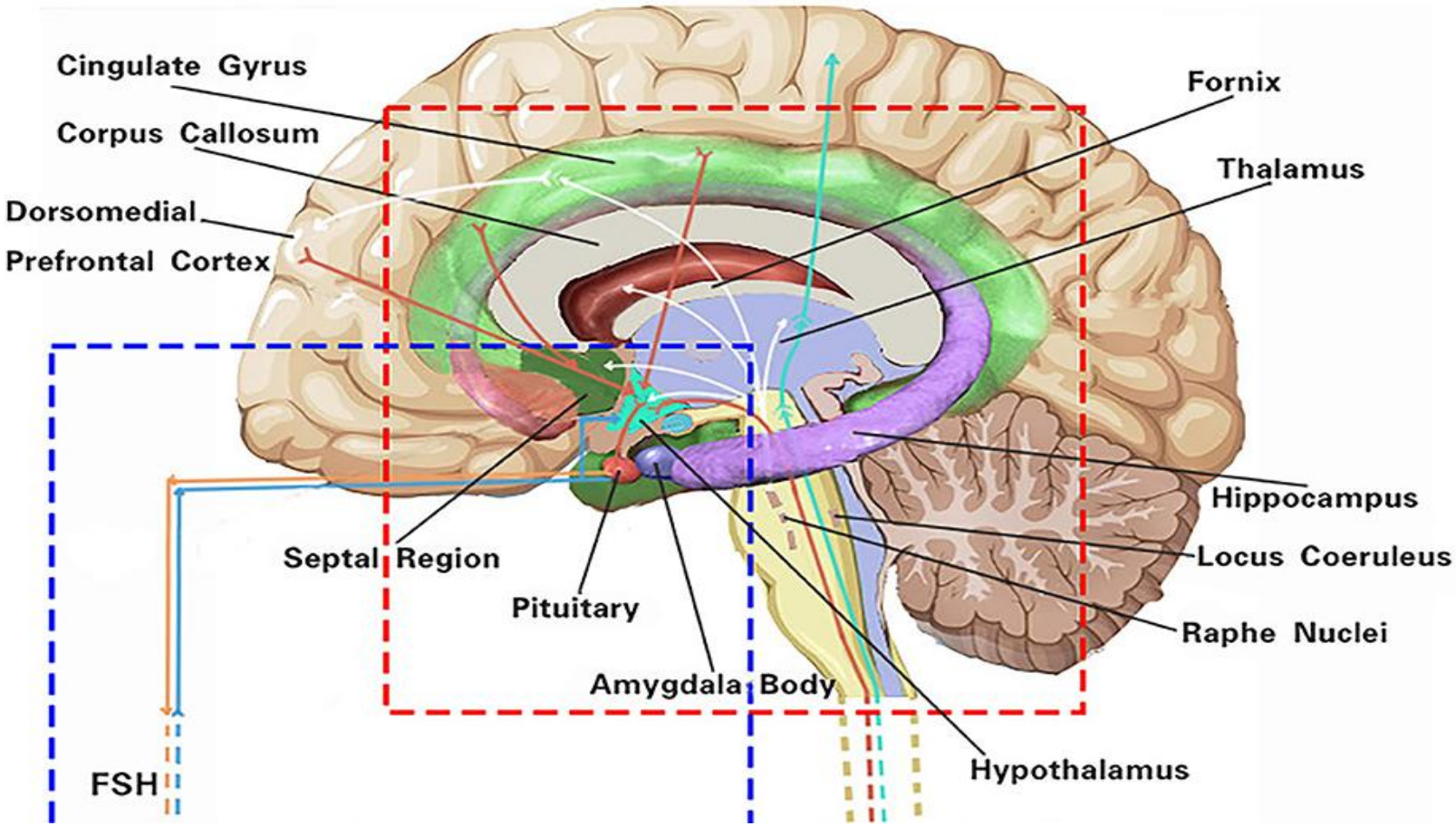
Motility	≥42%	•Abnormalities commonly seen in varicocele and primary ciliary dyskinesia patients, or infection. •Use of common antioxidants could be helpful in treatment of low sperm motility.	••Sperm motility and concentration provide more accurate information than morphology. ••19% of patients with clinical varicocele and infertility show isolated abnormalities in sperm motility.
Progressive Motility	≥30%	•Mitochondrial dysfunction causes reduced sperm progressive motility. •For each 10% reduction in progressive sperm motility there is nearly 11% hazard ratio for reduced pregnancy. •The presence of aneuploidies could be associated with reduced progressive motility.	•A significant negative correlation was observed between SDF and progressive motility. •A significant positive correlation was observed between vitamin E and β-carotene intake and progressive motility.

TMC	≥25.0 x10^6	•An average TMC of 10 million could be considered as the threshold value for decisions about treating a couple with IUI or IVF. •Low total motile sperm count (<5 million motile sperm after processing) on repeated SA -> IUI success rates may be reduced, and treatment with ART (IVF/ICSI) may be considered.	•TMC was suggested as a better indicator for the severity of male infertility than other methods. •IUI cycles with less than 10 million total motile sperm are significantly less likely to result in a pregnancy. •Microsurgical varicocelectomy can results in significant improvements in TMC.
TPMC	≥20.0 x10^6	•Post preparation TPMSC of at least 1 ×10^6/mL is minimum for IUI although some data indicates a low (<1%) chance of success with lower TPMSC.	•A post wash TPMC of <1.1 × 10^6 cells results in >25% risk of fertilization failure. •TPMC threshold of 50 million sperm is associated with the best predictive power to estimate earlier time-to-conception in couples.

Peroxidase Positive Cells	<1 $\times 10^6$ Cells /ml	•Elevated round cells on SA (>1million/mL) indicate increased WBCs or immature germ cells. •WBC may suggest genital tract infection or inflammation. •Immature germ cells are likely of no clinical significance. Specialized stains may then be used to differentiate the 2 cell types. •Consider a course of doxycycline, Bactrim, or ciprofloxacin.	•Peroxidase positive leucocytes are the main leucocytes present in semen. •WBCs may secrete cytokines and generate free radicals in the semen that may be detrimental to sperm function.

DNA fragmentation	<25%	•DNA fragmentation analysis is not recommended as initial evaluation. •In case of recurrent pregnancy loss, men should be evaluated with karyotype and SDF. •Some causes (i.e. anti-depressant use, genitourinary infection, varicoceles) are reversible. •2 or more ICSI failures using spermatozoa with uncorrectable high DNA fragmentation -> TESE and use of testicular spermatozoa for ICSI should be considered.	•Increased sperm DNA damage has been shown to result in male infertility and miscarriages. •ICSI was the first efficient ART option for men with high levels of DNA fragmentation. •Varicocelectomy can reduce altered seminal parameters single- and double-strand DNA fragmentation. •An increase in seminal reactive oxygen species levels by 25% was associated with a 10% increase in SDF.
TUNEL	<20%		
COMET assay	<14%	•Can consider SCSA, COMET assay, and TUNEL when plan for IUI, IVF, ICSI. •ICSI is the method of choice when DFI is higher than 30% detected by SCSA.	•DNA fragmentation measured by TUNEL assay is a highly valuable indicator of male fertility. •Varicocelectomy is associated with increased sperm DNA integrity determined by Comet assay. •SCSA evaluations determined men with DNA fragmentation ≥30% are at greater risk for low blastocyst rates.
SCSA	<30%		

Endocrinopathies in infertile men, implications, and treatments.



Lab	Reference range	High	Low
Total testosterone **Free testosterone** **Bioavailable testosterone**	300–1,080 ng/dL 47–244 pg/mL 200–682 ng/dL	•Endogenous causes include congenital adrenal hyperplasia, functional tumors (adrenal or testicular), and androgen insensitivity disorders. •Exogenous androgen use commonly present as oligospermia with motility and morphology abnormalities, and reduced gonadotropins. •IM T has been tested as male contraceptive with a 97% efficacy. •After stopping TRT, the sperm quality usually recovers within 4 mo. However, it might take up to 3 y in some cases.	•Klinefelter syndrome is the most common cause of primary testicular failure characterized by the combination of hypergonadotropic hypogonadism with gynecomastia, small testes. •Systemic diseases, certain medications (e.g. opiates or high-dose glucocorticoid), recreational drugs, eating disorders, and excessive exercise can transiently lower T levels. •SERMs such as clomiphene citrate and tamoxifen, block the suppressive action of E2 and stimulates gonadotropin secretion.
SHBG	11–80 nmol/L	•SHBG concentration increases by age usually beginning during the 3rd decade of life, which contributes to the reduced FT. •Other conditions associated with increased SHBG levels include cirrhosis and hepatitis, use of certain drugs (anticonvulsants, estrogens), and HIV.	•Metabolic syndrome is associated with lower SHBG, total, and FT levels. •SHBG levels decline with increase in BMI. •Other conditions associated with decreased SHBG levels include nephrotic syndrome, hypothyroidism, acromegaly, diabetes mellitus and use of certain drugs (glucocorticoids, progestins, and androgenic steroids).

Estradiol (E2)	10.0–42.0 pg/mL	•T to E2 (T: E2) is an important indicator of estrogen excess, and a goal ratio of greater than 10:1 is recommended for optimal spermatogenesis. •Underlying causes for high E2 levels include aromatase excess syndrome, estrogen-secreting tumors, occupational exposures, and exogenous sources (e.g. androgenic steroids). •In patients with T: E2 of less than 10:1, aromatase inhibitors can be used to decrease estrogen production.	•Decreased E2 is associated with increased sperm DNA damage. •Decreased E2 is associated with reduced sperm concentration and motility. •Standard treatment in hypogonadal men is treatment of T deficiency which corrects estrogen deficiencies through aromatization.
Prolactin	2.1–17.7 ng/mL	•Hyperprolactinemia can be caused by disruption of the hypothalamic dopaminergic inhibition or disturbance of portal circulation by sellar or parasellar tumors, first step is repeat prolactin and brain MRI. •Increased prolactin levels can result in spermatogenic arrest and impaired sperm motility.	•Hypoprolactinemia is associated with sexual dysfunction, higher prevalence of anxiety symptoms and arteriogenic ED. •Low levels of prolactin can result in lower sperm quality (oligozoospermia, asthenospermia), reduced function of seminal vesicles, and hypoandrogenism.
LH	1.7–8.6 IU/L	•Increased LH and T levels can be seen in androgen insensitivity disorders.	•Most commonly seen in men on exogenous T. •Hypogonadotropic hypogonadism could be because of an inherited and isolated deficiency (Kallmann syndrome) or may be acquired as part of diseases of the hypothalamic-pituitary area.
FSH	1.5–7.5 IU/L	•FSH serum concentration is increased with increasing severity of seminiferous epithelial destruction. •Klinefelter syndrome is the most common congenital etiology of hypergonadotropic hypogonadism and can be managed by TESE or micro-TESE with ICSI. •Gonadotropins, SERMs, AIs and a combination of these therapies are among the available medical treatment options for infertile men with hypergonadotropic hypogonadism.	•Evaluation of thyroid and adrenal function studies, serum prolactin, and cranial MRI should be performed to rule out structural abnormalities in patients with acquired secondary hypogonadis. •SERMs could be used as monotherapy in the men with hypogonadotropic hypogonadism to achieve normal semen parameters and subsequent pregnancy.

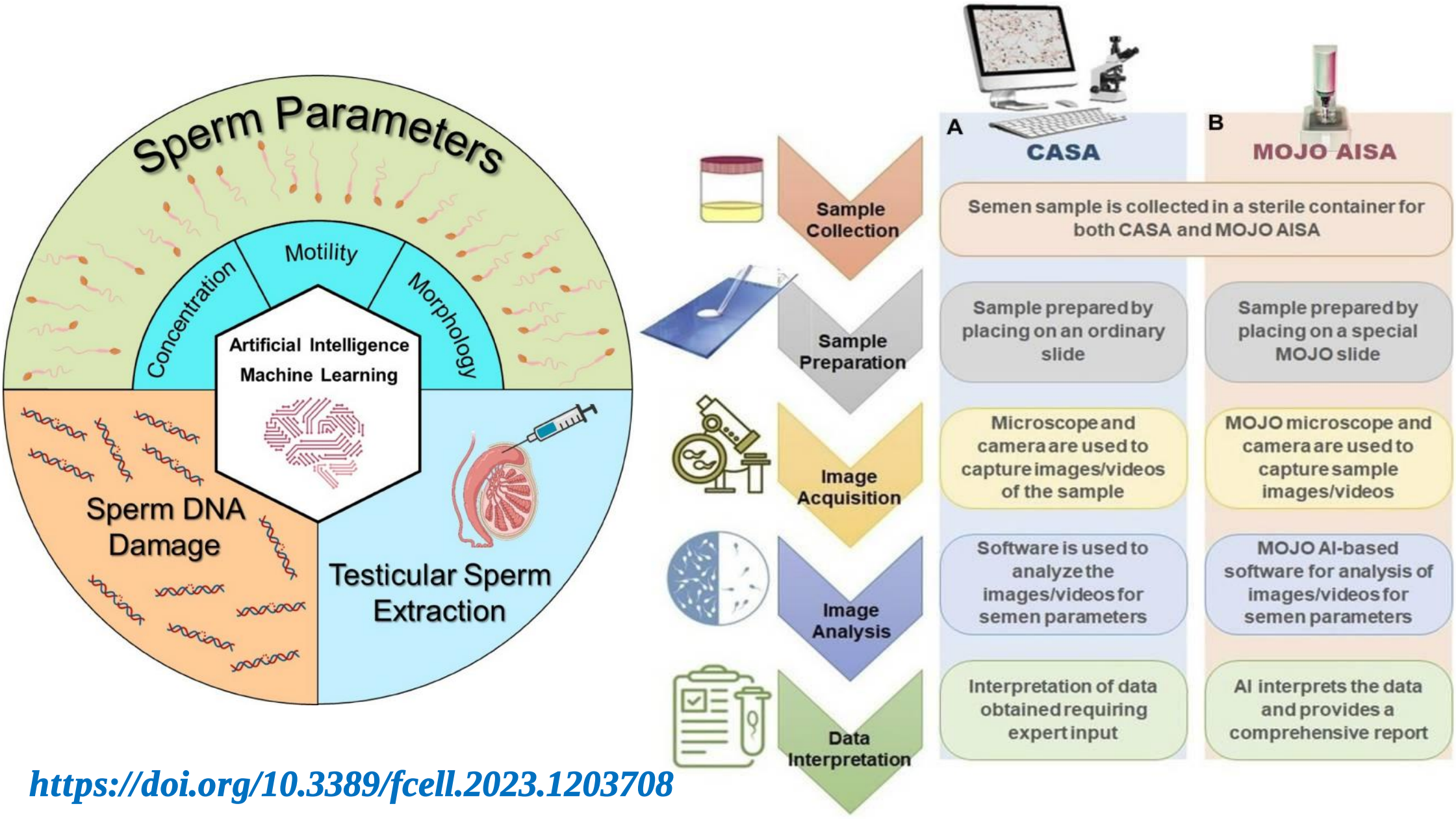
Genetic evaluation for the infertile male

Indication	Interpretation	Recent studies	
YCMD	NOA or <5 million/mL (AUA)	AZFa Deletion – SCOS/Azoospermia. Sperm retrieval not possible. Rec. Donor sperm AZFb Deletion – SCOS and/or maturation arrest with azoospermia. Sperm retrieval not possible. Rec. Donor sperm AZFc Deletion - presents various types of impaired spermatogenesis. Detection of sperm as high as 75%.	Kohn et. Al 2019. Meta-analysis estimated the prevalence of YCMD in azoospermia is approximately 8%–12%, whereas those in very severe oligozoospermia (0–1 million sperm/mL), severe oligozoospermia (1–5 million sperm/mL), and sperm concentrations of 5–20 million sperm/mL are approximately 5%, 0.8%, and 0.5%, respectively. Indicating that YCMD is much less common than previously reported in men with >1 to 5M/ml sperm.
Karyotype	NOA or <5 million/mL (AUA)	Klinefelter Syndrome – 1/660 men. Most common cause of NOA. (47, XXY) and variants such as (47,XXY/46,XY). Translocations and inversions are found in up to 10% of infertile men and are the most frequent causes of oligospermia. Only large chromosomal abnormalities such as aneuploidies, Robertsonian and balanced translocations, and inversions can be detected. KA only detects abnormalities <5Mb in size.	Dul et al. 2010 investigated the incidence of chromosomal abnormalities and showed that azoospermia occurred in 15.4% of infertile men, whereas 0–1, 1–5, 5–20, and more than 20 million sperm/mL were detected in 3.0%, 2.1%, 2.7%, and 2.9% of cases, respectively. These findings suggest that sperm concentration may not be the most effective threshold for recommending karyotype analysis.
CFTR	CBAVD or CUAVD (AUA/EAU) Female partners should also be tested	CBAVD - Bilateral absence occurs in 2–10% of all infertile men but accounts for up to 40% of obstructive azoospermia cases. *CFTR* is a 250 Kb gene on chromosome 7 which encodes an ATP-dependent chloride channel found primarily in epithelial cells. Over 2,000 mutations have been described with varying phenotypic consequences.	Yang et al. 2019 Meta-analysis showed that the IVS8-5T mutation may be positively associated with the risk of nonobstructive azoospermia (odds ratio [OR] 1.69; 95% CI: 1.12–2.55), not just obstructive secondary to CBAVD.

G-protein coupled receptor G2 (ADGRG2)	No current guideline.	Consider testing in men with CBAVD, without CFTR mutation, and no kidney anomalies.	Patat et al. 2016 performed whole exome sequencing on 12 men with obstructive azoospermia without CFTR mutations or kidney abnormalities. Loss of function mutations in ADGRG2 were identified in 3 men. Yuan et al. 2019 evaluated 72 patients with congenital absence of the vas deferens and compared them with 224 healthy fertile controls. They found 2 novel variants in ADGRG2 in men without CFTR mutations.
Testis-expressed gene 11 (TEX11)	No current guideline	Xp11. Mutations in TEX11 are among the most frequently identified genetic mutations in the general idiopathic NOA population; however, they are only identified in 1% to 2.4% of cases.	Yatsenko et al. 2015, Propose that restricting screening to NOA men with meiotic arrest increases the detection of likely deleterious mutations in TEX11 from 2.4% to 15%, and may support TEX11 sequencing specifically in this population in the future.
DEAD-box helicase 3 Y-linked (DDX3Y)	No current guideline	DDX3Y encodes a conserved ribonucleic acid helicase specifically expressed in premeiotic spermatogonial cells and is mapped within the AZFa region. Deletion causes SCOS.	Foresta et al. 2000, DDX3Y expression is required for human fetal germ-cell proliferation, and DDX3Y deletions have been identified in patients with SCOS or severe oligospermia.
Doublesex and Mab-3 related transcription factor 1 (DMRT1)	No current guideline	DMRT1 encodes a testis-specific transcription factor essential for testis differentiation. Deletion of chromosome 9p, which includes DMRT1, is known to be associated with 9p deletion syndrome and XY gonadal dysgenesis.	Lopes et al. 2013 Smaller deletions and mutations of this gene have been identified in patients with NOA without XY gonadal dysgenesis.
Dynein Axonemal Heavy Chain 1 (DNAH1)	No current guideline	DNAH1 encodes an axonemal inner dynein arm heavy chain, which often lacks the central pair (9+0 structure). Mutations in the DNAH1 gene are responsible for 25% of MMAF, which are episodes of asthenoteratospermia caused by flagella abnormalities.	Collodel et al. 2006, Flagella abnormalities are associated with an elevated frequency of aneuploidy and poor IVF-ICSI outcomes. Khelifa et al. 2014, proposed that the lack of DNAH1 leads to a disorganized axoneme and it is this structural alteration that may be linked to a decreased implantation rate.

READY FOR TESTING
PLEASE ENTER DATA INTO SQA-VISION
SQA-VISION
Power
1
2
3
4
5
6
7
8
9
0
.
F
I-Button
Service
In
Zoom
Out
Enter
Esc
Delete
"Remember. it All Started
...With A Sperm!"

AndroVision
38.0 38.0
HTi



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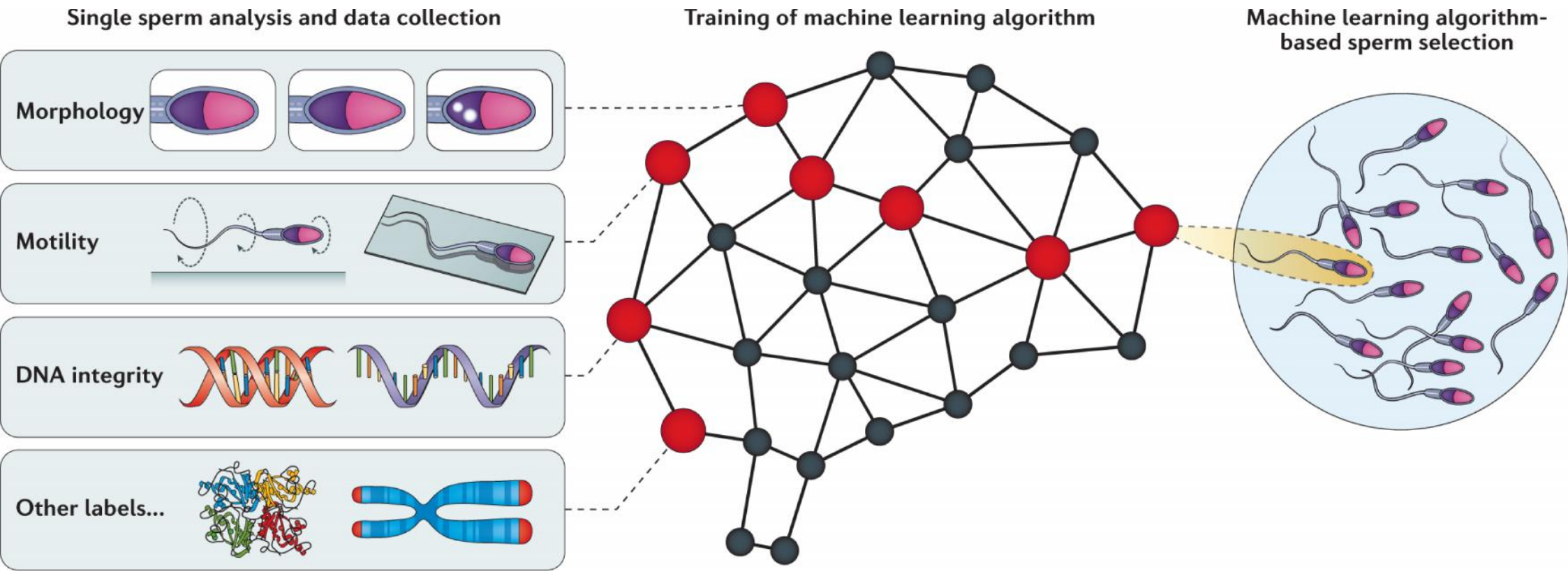

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AI STATION

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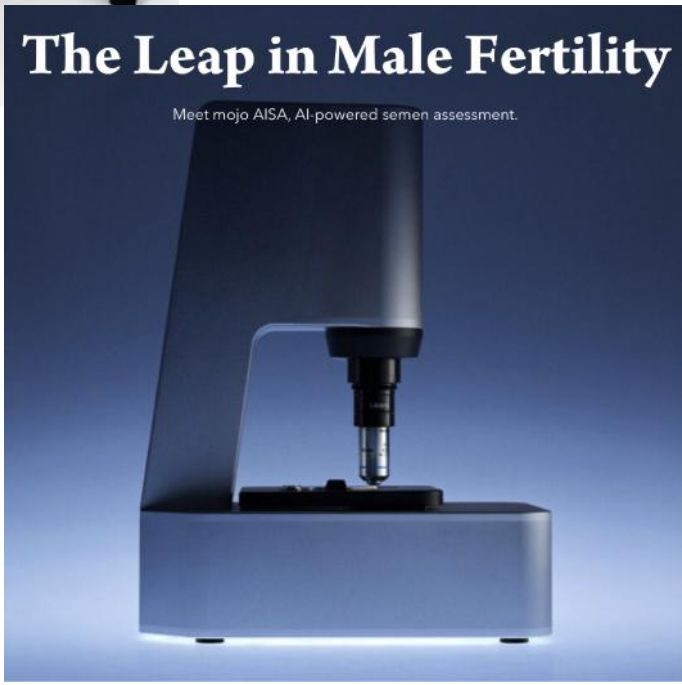
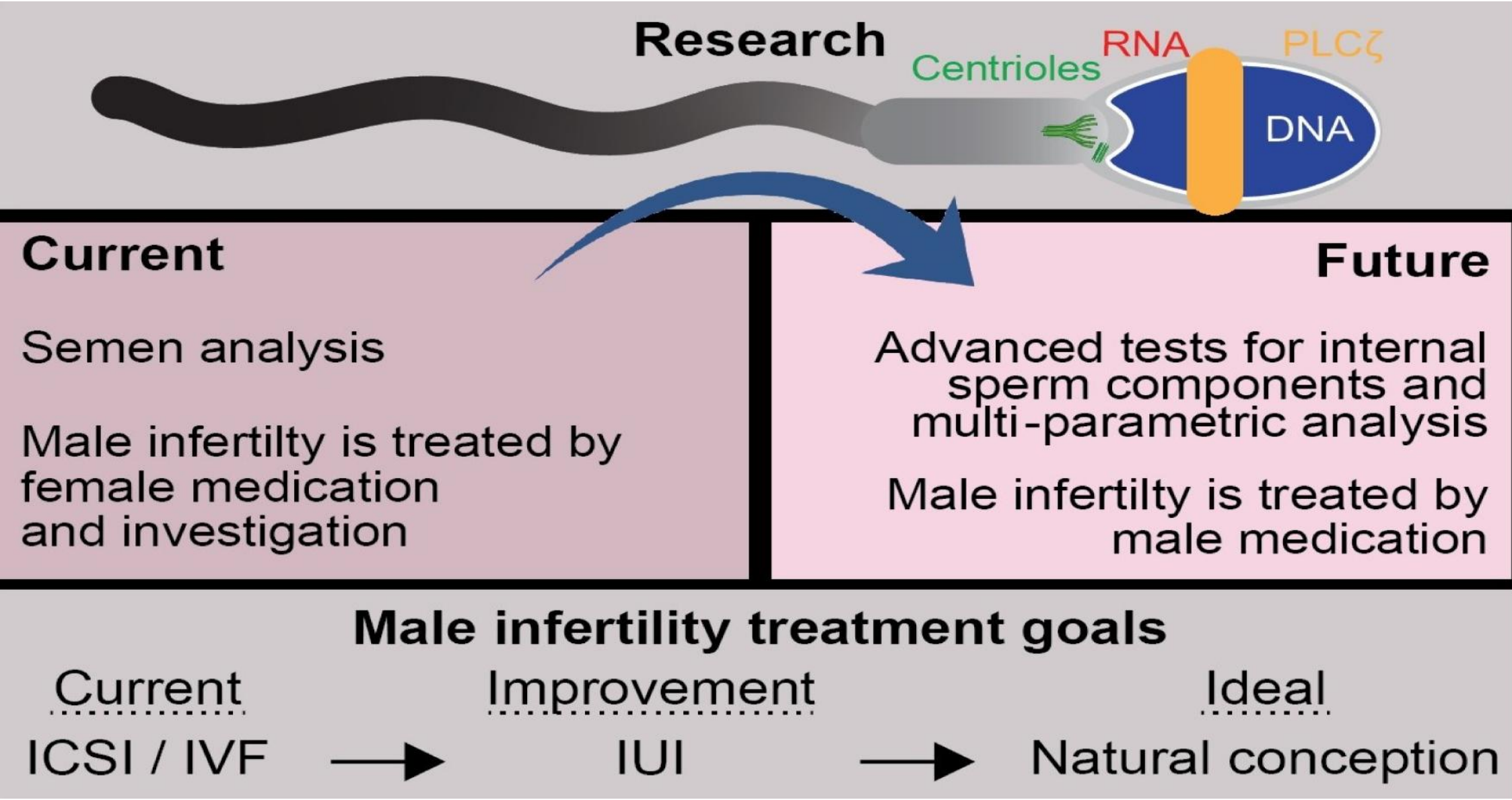
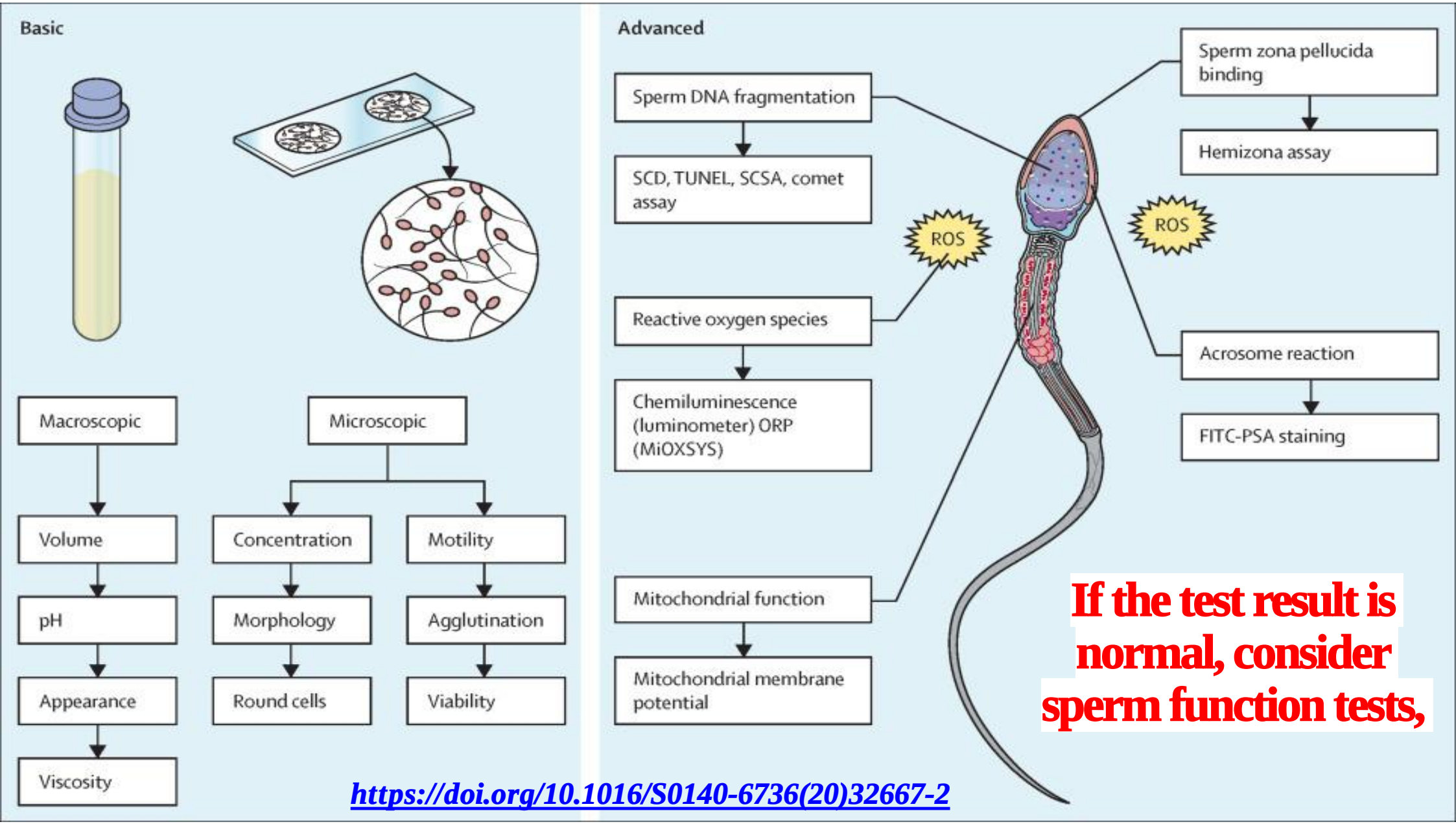




Cells **2020**, *9*(4), 990; **https://doi.org/10.3390/cells9040990**



If the test result is normal, consider sperm function tests,

https://doi.org/10.1016/S0140-6736(20)32667-2

THANKS FOR YOUR ATTENTION