BIOMARKERS IN HUMAN REPRODUCTION

Lesson 4

2023/2024

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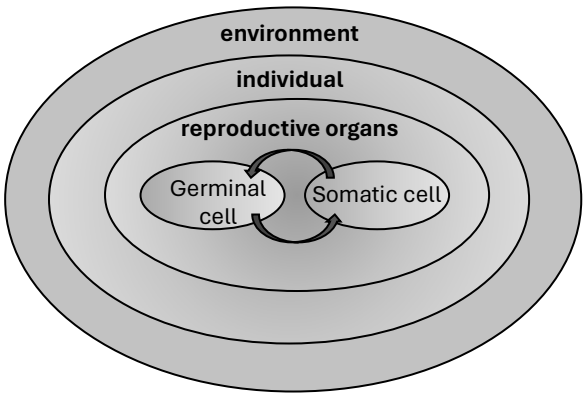
The **success of fertilization** depends largely on the fertility potential of the **gametes**, and therefore the quality of both the **sperm** and the **egg** are equally important.



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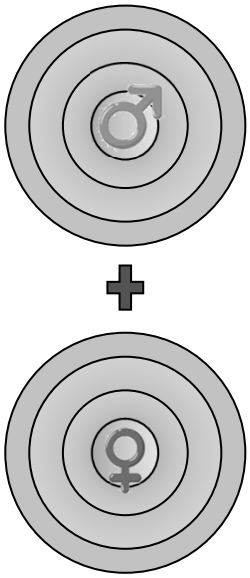
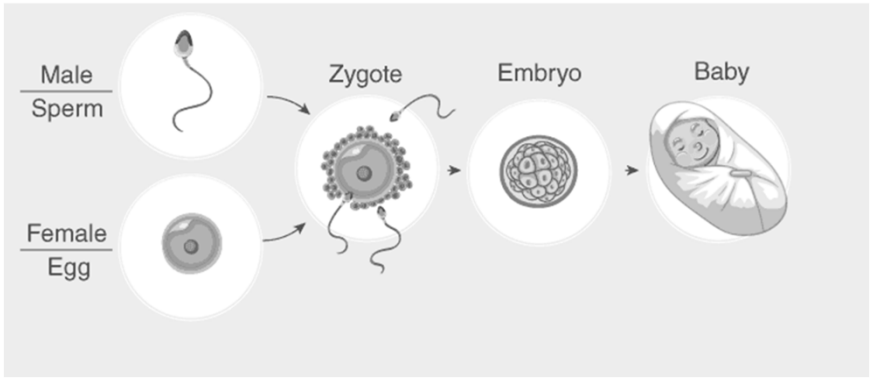
Reproduction

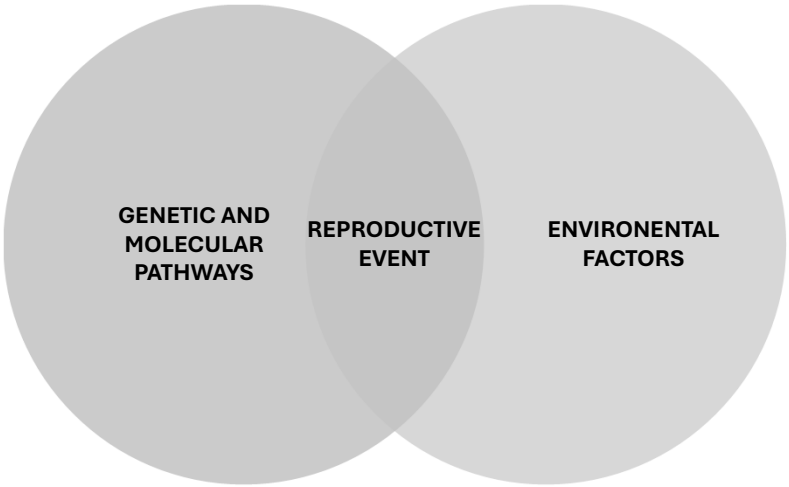




3

Reproduction

Lifestyle factors affect fertility in women and in men



- **nutrition**
- **weight**
- **exercise**
- **physical and psychological stress**
- **environmental and occupational exposures**
- **substance and drug use and abuse**
- **medications**

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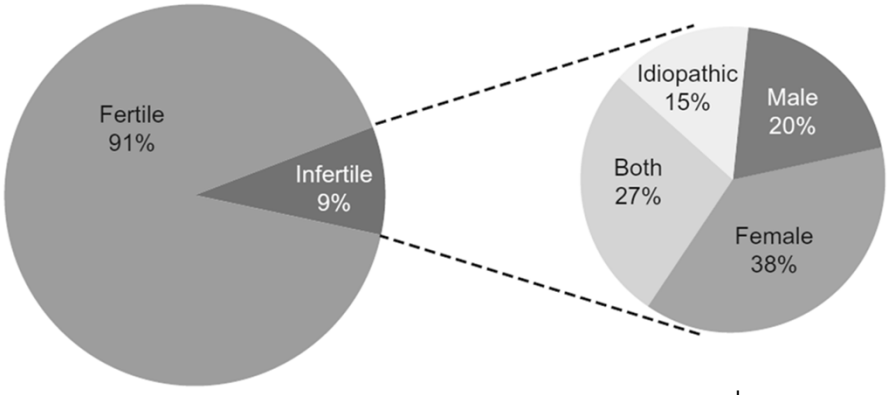

- woman and man age,
- frequency of sexual intercourse,
- exercise, obesity,
- use of drugs or medications,
- smoking,
- infection,
- previous abdominal surgery,
- occupational and environmental exposure to toxicants,
- psychological factors,
- stress

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For example…

Obesity is linked to lower sperm count and quality in men.

Among **obese** women who have polycystic ovary syndrome (PCOS), losing 5% of body weight greatly improves the likelihood of ovulation and pregnancy.

Being **underweight** is linked to ovarian dysfunction and infertility in women.

Strenuous **physical labor** and taking multiple **medications** are known to reduce sperm count in males.

Excessive exercise is known to affect ovulation and fertility in women.

Research shows that using body-building **medications** or **androgens** can affect sperm formation.

Substance use, including smoking **tobacco**, using other tobacco products, **marijuana** use, **heavy drinking**, and using illegal drugs such as **heroin** and **cocaine** reduce fertility in both men and women.

Having **high blood pressure** changes the shape of sperm, thereby reducing fertility.

Radiation therapy and **chemotherapy** can cause infertility in females and males

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Biomarkers: purposes in reproductive medicine

1. **biomarkers of a disease or developmental stage**: some biomarkers serve as indicators of specific phases in normal and abnormal developmental processes;
2. **biomarkers of effect**: physical or chemical environmental exposures can generate an assembly of localized or systemic effects and can be measured at the molecular, cellular, or clinical level;
3. **biomarkers of exposure**: biomarkers can be used to identify potential toxic exposures because of significant changes in biological function or appearance that signify exposure to a specific stimulus of a biological, physical, or chemical nature;
4. **biomarkers of susceptibility**: genetic biomarkers may also be used to identify predisposition to develop specific conditions.

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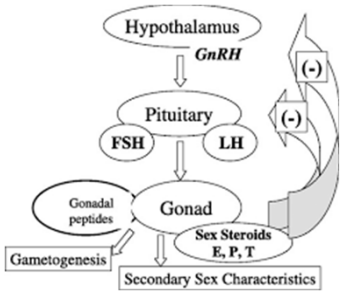
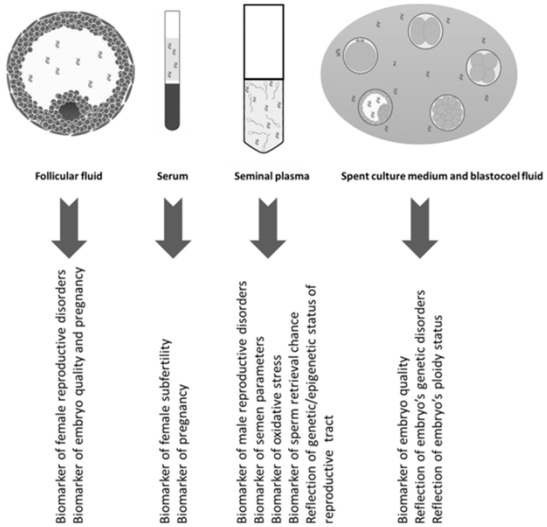
Biomarkers of reproductive function:

- Sex steroid hormones
- Protein hormones
- Endogenous modulatory biomarkers of reproductive function





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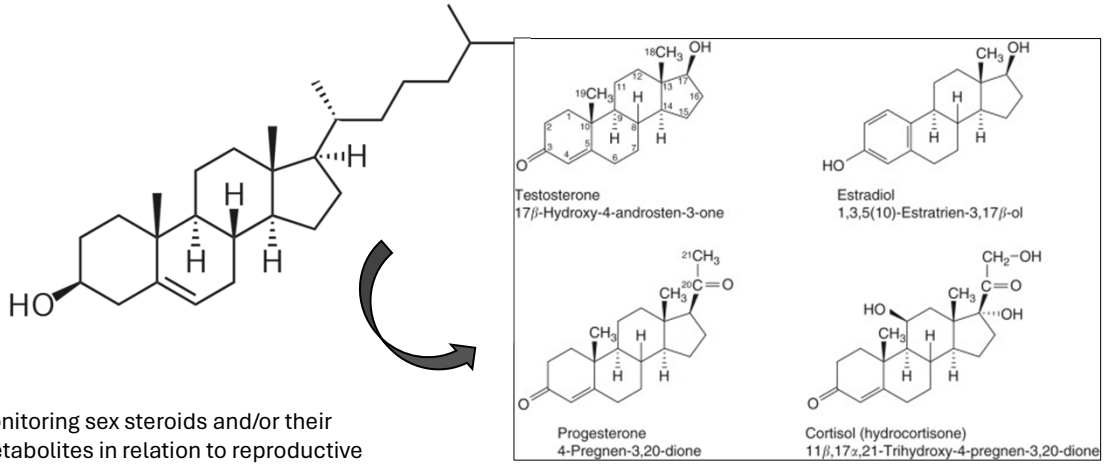
Sex steroid hormones



Monitoring sex steroids and/or their metabolites in relation to reproductive function has been an effective tool in a wide range of wildlife research of males and females

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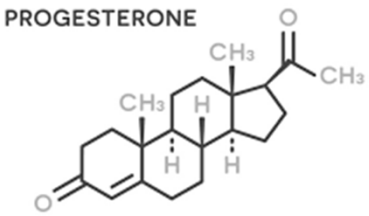
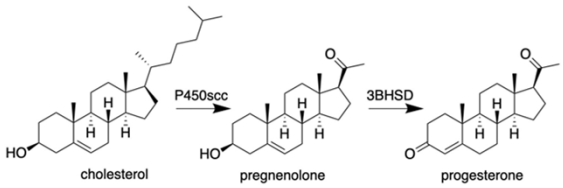
Sex steroid hormone



- Progesterone is a female sex steroid hormone
- It is synthesized in the corpus luteum of the ovaries
- The blood level of progesterone varies with the menstrual cycle
- The hormone prepares the uterus for a possible pregnancy
- Following fertilization, the placenta also start to synthesize progesterone in order to maintain the pregnant state
- The development of the mammary glands is also stimulated by progesterone.

Formula = $C_{21}H_{30}O_2$
Molecular weight = 314.46 g/mol



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Progesterone

- Pregnancy hormone
- Its presence in the blood was confirmed in 1958.
- Its level rises from less than 0.1 µg/100mL (nonpregnant) up to 87 µg/100 mL during pregnancy.
- Low progesterone levels have been reported in cases of premature births.
- It keeps the uterus quiet during pregnancy, reduces the mother's immune response and supports the implantation of the fetus in the uterus.
- A decrease in progesterone levels facilitates the onset of labor and stimulates milk production. Lower progesterone concentrations than normal are the leading cause of premature labor, and this can be prevented by administering progesterone to pregnant women.

Serum progesterone is a potential biomarker for differentiating nonviable pregnancies from viable pregnancies.
Progesterone + human chorionic gonadotropin =improves the specificity of monitoring **fetal viability** during early gestation.
Receptors of estrogen and progesterone act as prognostic biomarkers of **ovarian cancer**.

Phase	Progesterone level
Baseline (follicular phase of menstrual cycle)	0.15 to 0.7 nl/mL
Baseline (luteal phase of menstrual cycle)	2 to 25 ng/mL
Pregnancy, 1st trimester	7.25 to 44 ng/mL
Pregnancy, 2nd trimester	19.5 to 82.5 ng/mL
Pregnancy, 3rd trimester	65 to 229 ng/mL

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Protein hormones

- Anti-Müllerian hormone (AMH)
- Follicle stimulating hormone (FSH)
- Inhibin
- Luteinizing hormone (LH)
- Oxytocin
- Prolactin
- Relaxin
- Prostaglandin

Endogenous modulatory biomarkers of reproductive function

- Metabolism ⟹
  - ❖ Thyroid hormones
  - ❖ Leptin and insulin
  - ❖ Glucocorticoids
- Melatonin
- Markers of oxidative stress
- MicroRNAs (miRNA)
- Microbiome
- Immune function

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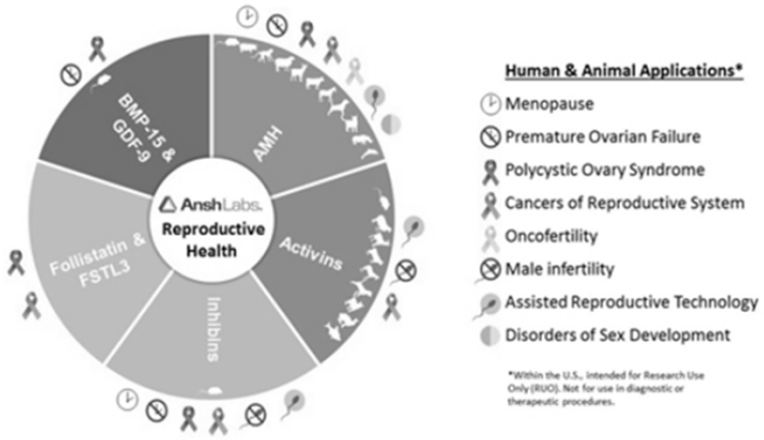

https://www.anshlabs.com/news/emerging-biomarkers-for-reproductive-health-offered-by-ansh-labs/#POF

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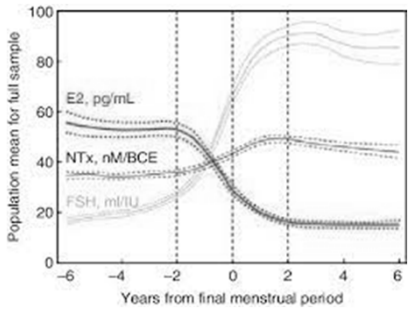
MENOPAUSE



- Traditionally **FSH** is measured to diagnose menopause. FSH, secreted from the anterior pituitary gland offers an indirect measure of ovarian reserve and is subject to cycle variability. Hormones produced from the ovary serve as direct markers of ovarian reserve. AMH and Inhibin B levels have been shown to decline approaching menopause and numerous studies report correlation of AMH decline with onset of menopause complications.
- Accurate prediction of the final menstrual period will be tremendously advantageous for physicians to decide a proper hormone replacement therapy to ameliorate vasomotor symptoms, decide appropriateness of cessation of contraception, make recommendations for bone mineral density (BMD) measurement early on to identify patients at high risk of losing bone at an accelerated rate leading to osteoporosis, identify those early menopause onset women with increased risk of breast cancer and cardiovascular or stroke event risk, etc.

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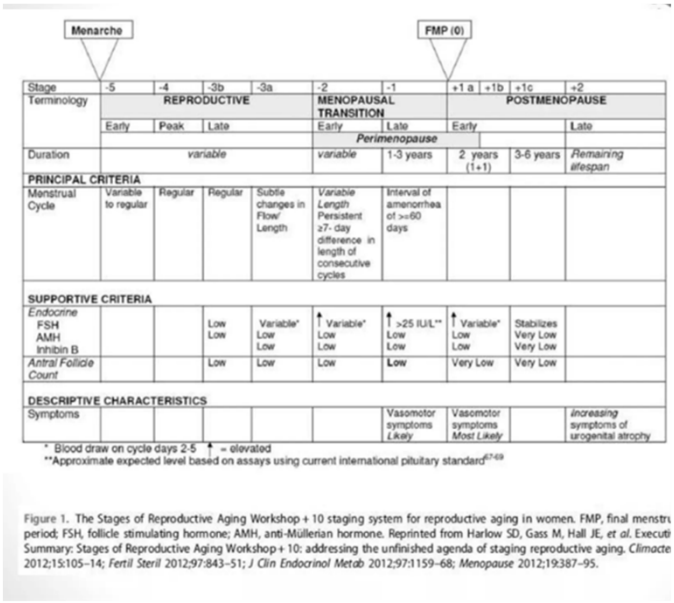
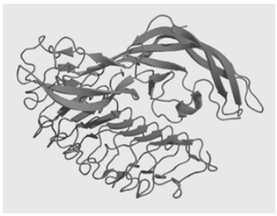
***Follicle stimulating hormone* FSH**



Stage	-5	-4	-3b	-3a	-2	-1	+1 a	+1b	+1c	+2
Terminology	REPRODUCTIVE				MENOPAUSAL TRANSITION		POSTMENOPAUSE			
	Early	Peak	Late		Early	Late	Early			Late
	Perimenopause									
Duration	variable				variable	1-3 years	2 years (1+1)	3-6 years		Remaining lifespan
PRINCIPAL CRITERIA										
Menstrual Cycle	Variable to regular	Regular	Regular	Subtle changes in Flow/ Length	Variable Length Persistent ≥7- day difference in length of consecutive cycles	Interval of amenorrhea of >=60 days				
SUPPORTIVE CRITERIA										
Endocrine FSH AMH Inhibin B			Low Low	Variable* Low Low	↑ Variable* Low Low	↑ >25 IU/L** Low Low	↑ Variable* Low Low	Stabilizes Very Low Very Low		
Antral Follicle Count			Low	Low	Low	Low	Very Low	Very Low		
DESCRIPTIVE CHARACTERISTICS										
Symptoms						Vasomotor symptoms Likely	Vasomotor symptoms Most Likely			Increasing symptoms of urogenital atrophy

* Blood draw on cycle days 2-5 ↑ = elevated
**Approximate expected level based on assays using current international pituitary standard[47-49]

Figure 1. The Stages of Reproductive Aging Workshop + 10 staging system for reproductive aging in women. FMP, final menstru period; FSH, follicle stimulating hormone; AMH, anti-Müllerian hormone. Reprinted from Harlow SD, Gass M, Hall JE, *et al.* Executi Summary: Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. *Climacte* 2012;15:105–14; *Fertil Steril* 2012;97:843–51; *J Clin Endocrinol Metab* 2012;97:1159–68; *Menopause* 2012;19:387–95.



FSH is a glycoprotein produced by gonadotrophic cells in the anterior pituitary in response to pulsatile secretion of hypothalamic GnRH and granulosa cell derived activin.

FSH receptors are found on granulosa cells within developing preantral follicles and stimulation of these receptors promotes folliculogenesis.

FSH specifically acts on granulosa cells to convert testosterone, produced by neighboring theca interna cells, into estradiol (E2) by increasing the activity of aromatase.

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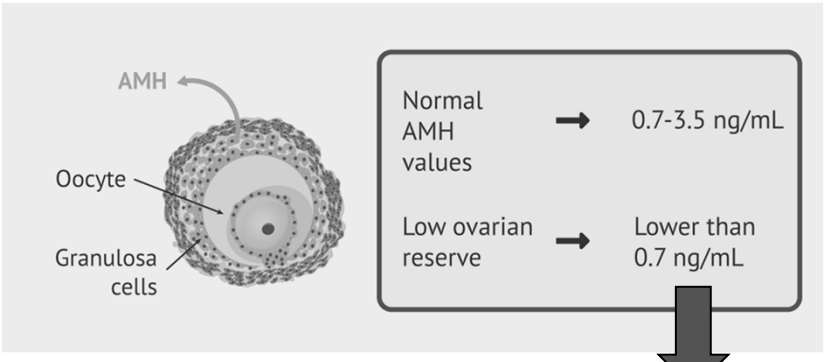
AMH, Anti-Müllerian hormone
has beenproven to be a sensitive biomarker
for detecting the menopausal transition



early ovarian aging.

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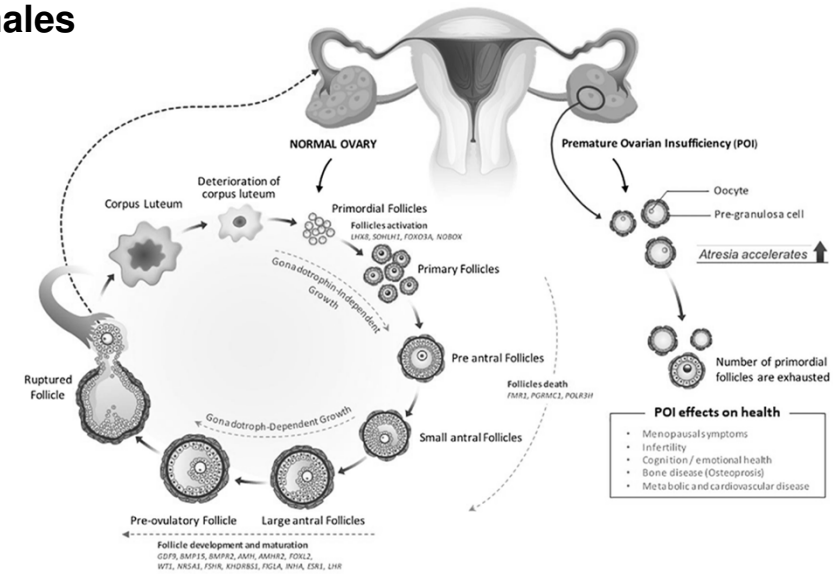
Biomarkers of common reproductive impairments in females



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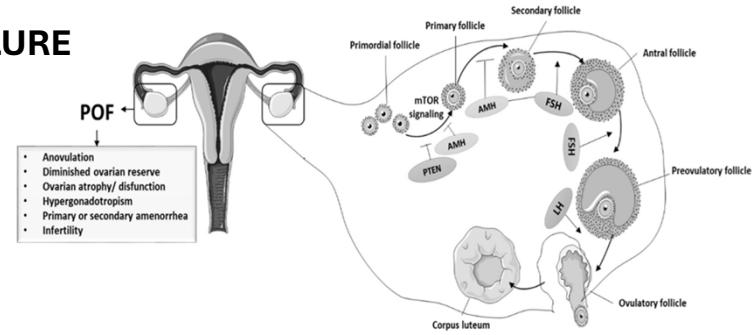
PREMATURE OVARIAN FAILURE (POF)

About 15% of the female population faces infertility issues because of premature ovarian failure



POF is defined as loss of function of ovaries before age 40.

Underlying causes range from chromosomal defects to chemotherapy induced gonadal toxicity.

AMH and Inhibins applicable to menopause diagnosis may also be useful in the diagnosis of POF leading to early onset menopause.

Numerous studies associate mutations in BMP-15 and GDF-9 with POF.

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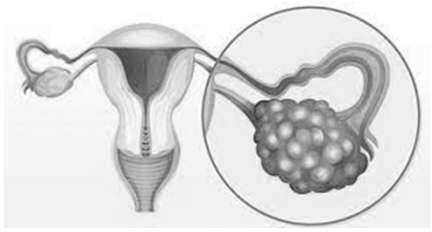
POLYCYSTIC OVARY SYNDROME (PCOS)



PCOS is a complex endocrine system disorder with reproductive, metabolic, cardiovascular and psychological manifestations.

PCOS is a significant medical problem.

Menstrual history and ultrasonography along with serum-based markers like testosterone and LH to FSH ratio are currently used to diagnose PCOS. However, these methods are error-prone and are at best suggestive of PCOS.

AMH levels are reported high in PCOS patients and in recent years has gained considerable attention as a potential diagnostic tool.
There is a tremendous need to address these limitations to improve patient quality of life from menarche through menopause by encouraging the early diagnosis of PCOS with effective assays and diagnostic standards.

Biomarkers:

↑ levels of miR-21 (Serum)

↑ levels of AMH + ↓ low levels of SHBG (sex hormone-binding globulin)

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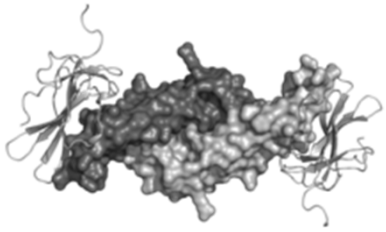
Anti-Müllerian hormone (AMH)



Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β (TGF-β) family.

During embryonic development, AMH secreted by Sertoli cells in the developing testis causes regression of the Müllerian or paramesonephric ducts and inhibits the formation of the female duct system.

In the female, AMH is produced by granulosa cells in follicles, with expression highest in the preantral and small antral follicles.

AMH is a marker of fertility in women, cattle, goats, pigs, and mice, with circulating concentrations positively correlated to the number of morphologically healthy oocytes in the follicular reserve.

In the human, serum AMH is used to determine the onset of menopause, a state of reproductive cycle cessation due to ovarian follicle depletion.

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CANCERS OF REPRODUCTIVE SYSTEM

The five main types of gynecologic cancer are:

- **cervical**
- **ovarian**
- **uterine**
- **vaginal**
- **vulvar**

Besides their utility in assessment of ovarian reserve, many TGF-beta superfamily proteins may also be useful in researching various cancers of the reproductive system.

Positive correlation between AMH and breast cancer risk in premenopausal women.

AMH was also reported as a biomarker for adult-type granulosa cell tumors. Utility of AMH as a biomarker in cancer research is further supported by animal studies where serum AMH levels were used to diagnose canine Sertoli cell tumors and granulosa cell tumors in mares.

Additionally, several studies highlight the importance of Inhibin A, Inhibin B, Follistatin and Follistatin-Like 3 as biomarkers in cancers such as granulosa cell tumors, breast cancer, and ovarian cancer.

doi: 10.5491/SHAW.2012.3.3.166

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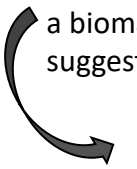
Biomarkers of reproductive cancers

1. **Follistatin:** is a glycoprotein present in the follicular fluid of the ovary. It serves as a biomarker of ovarian mucinous tumors and pregnancy. Another study suggested its use in the diagnosis of lung adenocarcinoma



support the survival of adenocarcinoma cells by neutralizing the action of activin A.

Follistatin Gene Therapy Improves Ambulation in Becker Muscular Dystroph

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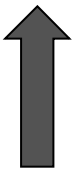
Biomarkers of reproductive cancers

2. Cancer antigen 125: Ovarian carcinoma is diagnosed by CA-125. It is a tumor-associated antigen released by the coelomic epithelium that serves as a useful biomarker for endometriosis. It is secreted in bodily fluids in soluble form or expressed on cell surfaces that experience metaplastic differentiation into Müllerian-type epithelium.

CA-125:	benign conditions:	uterine fibroids endometriosis during early pregnancy
	malignant conditions:	non-Hodgkin lymphoma Mesothelioma Leiomyosarcoma breast cancer gastric cancer liver diseases ovulatory cycles congestive heart failure tuberculosis

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Biomarkers of reproductive cancers

3. Interleukin-8: Interleukin-8 is an important contributing factor to male genital tract inflammation/infection.
It is associated with benign prostatichyperplasia-related inflammation. Seminal plasma interleukin-8 may serve as a predictive and reliable surrogate marker of prostatitis and leukocytospermia.

It has also been reported that seminal plasma interleukin-8 is involved:
in the swelling of the prostate, along with other organs of the male reproductive tract, especially the epididymis and seminal vesicles, but not the testis.

Interleukin-8 has also been found to be a promising marker for several clinical conditions:
- non-Hodgkin lymphoma
- nosocomial bacterial infections
- osteomyelitis,
- inflammatory bowel disease,
- acute pyelonephritis
- pulmonary infections
- vesicoureteral reflux
- Prostatitis
- urinary bladder cancer

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Biomarkers of reproductive cancers

4. Interleukin-6: Interleukin-6 is a multifunctional cytokine that acts as a triggering factor of B-lymphocytes because it triggers the differentiation of B cells, which give rise to antibody-producing plasma cells.

Interleukin- 6 affects the progression of prostate cancer and prostate carcinoma, as well as the production of prostate-specific antigen.

Menstrual effluents contain interleukin-1β, interleukin-6, and tumor necrosis factor α, thereby acting as a biomarker of chronic endometritis, and it has been reported that the tumor necrosis factor α gene is more highly expressed in women with endometriosis.

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Cancer site	Agent	IARC classification‡	Sufficient evidence for cancer in humans	Limited evidence for cancer in humans
Cervix uteri	Diethylstilbestrol (*in utero* exposure)	Group 1	X	
	Estrogen-progestogen oral contraceptives	Group 1	X	
	Human immunodeficiency virus type 1	Group 1	X	
	Human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	Group 1	X	
	Tobacco smoking (active)	Group 1	X	
	Human papillomavirus types 26, 53, 66, 67, 68, 70, 73, 82	Group 1		X
	Tetrachloroethylene	Group 2A		X
Corpus uteri	Estrogen hormone replacement therapy	Group 1	X	
	Estrogen-progestogen hormone replacement therapy	Group 1	X	
	Tamoxifen	Group 1	X	
	Diethylstilbestrol	Group 1		X
Ovary	Asbestos (all forms)	Group 1	X	
	Estrogen hormone replacement therapy	Group 1	X	
	Tobacco smoking (active)	Group 1	X	
	Talc-based body powder (perineal use)	Group 2B		X
	X-radiation, γ-radiation	Group 1		X
Vulva	Human papillomavirus type 16	Group 1	X	
	Human immunodeficiency virus type 1	Group 1		X
	Human papillomavirus types 18, 33	Group 1		X
Vagina	Diethylstilbestrol (*in utero* exposure)	Group 1	X	
	Human papillomavirus type 16	Group 1	X	
	Human immunodeficiency virus type 1	Group 1		X

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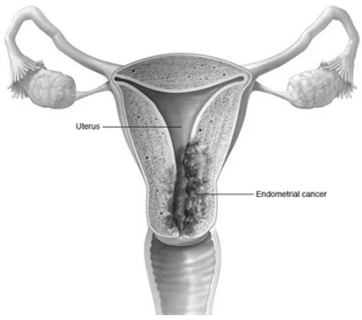
Endometrial cancers ⟹ is one of the most common gynecological malignancies

Biomarkers:

↑ Oncofetal protein IMP3*

↑ methylation of SOX1, HS3ST2, and AJAP1



*Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is an oncofetal protein highly expressed in fetal tissue and malignant tumors but rarely found in adult benign tissues.

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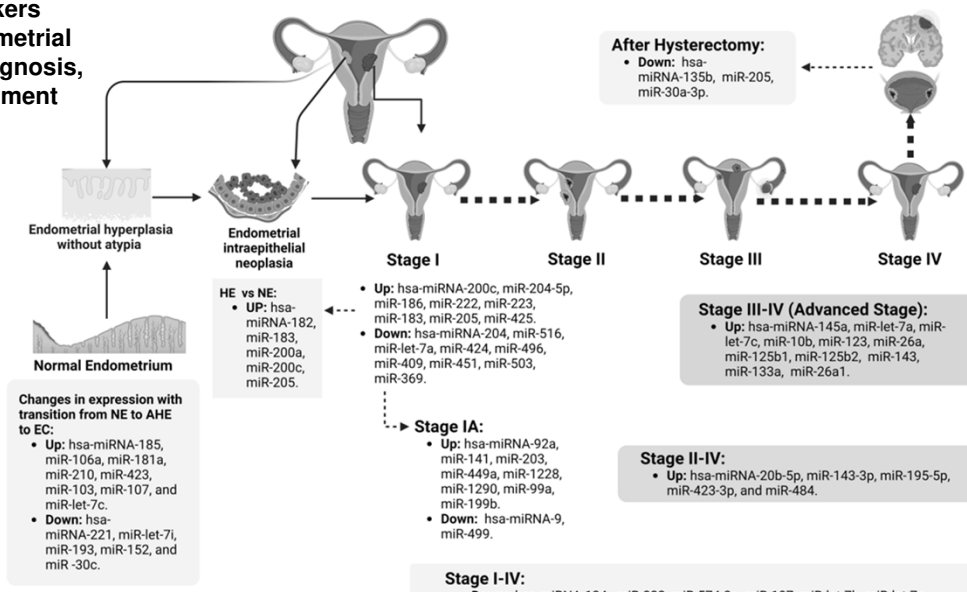
Circulating and Endometrial Tissue microRNA Markers Associated with Endometrial Cancer Diagnosis, Prognosis, and Response to Treatment



https://doi.org/10.3390/cancers15102686

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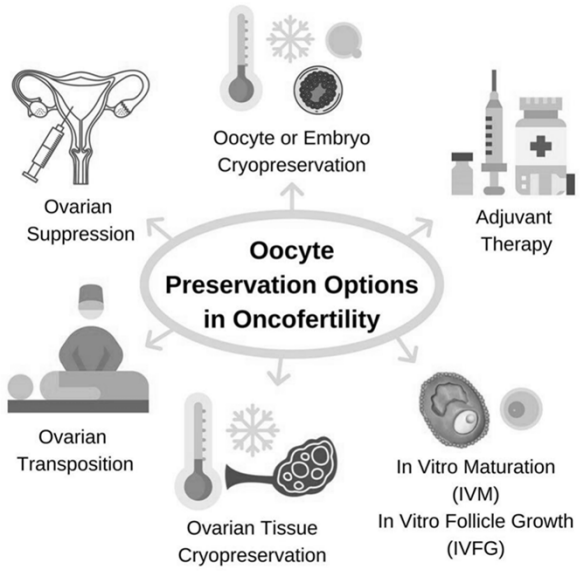
ONCOFERTILITY



Several cancers and the treatment regimens adversely affect gonadal health, thus preserving the reproductive future is a major emotional and financial concern among cancer survivors.

Numerous studies show the promise of **AMH as a serum-based biomarker** for the assessment of ovarian reserve in cancer patients and survivors. It is important to note that assessment of ovarian reserve in certain cancer patients and survivors involves measuring very low AMH levels.

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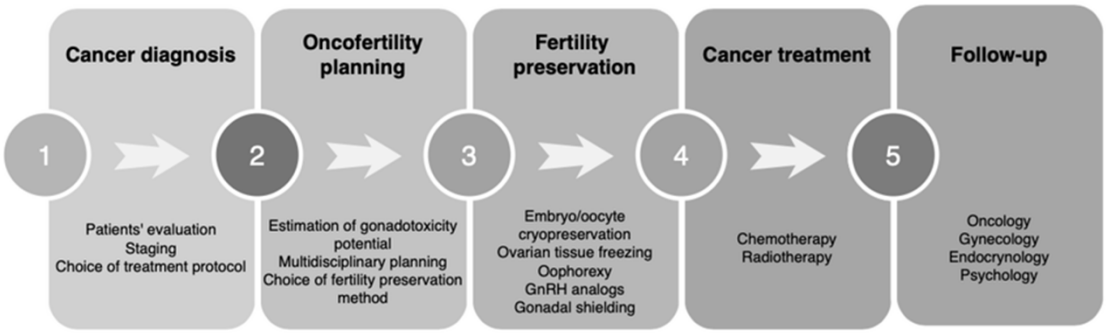

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Option for fertility preservation in girl undergoing cancer treatment

Method		Can It Be Used in Prepubertal Girls?	Does It Cause Any Treatment Delay?	Does It Involve a Surgical Procedure?	Success Rate
Established methods	Embryo cryopreservation	No	Yes	Yes	Live birth rate 27.7% per frozen embryo [43]
	Oocyte cryopreservation	No	Yes	Yes	Live birth rate of 3–6% per frozen oocyte [43]
Experimental methods	Ovarian tissue freezing and transplantation	Yes	No	Yes	Live birth rate 32% per transplant; endocrine recovery rate was 93% [45]
	Oocyte in vitro maturation	Maybe	No	Yes	21.5–55.6% per cycle [46]
Debatable methods	GnRH analogs	No	No	No	Debatable
	Oophoropexy	Yes	No	Yes	66–79% of ovarian function preservation [47,48]
	Gonadal shielding	Yes	No	No	Debatable

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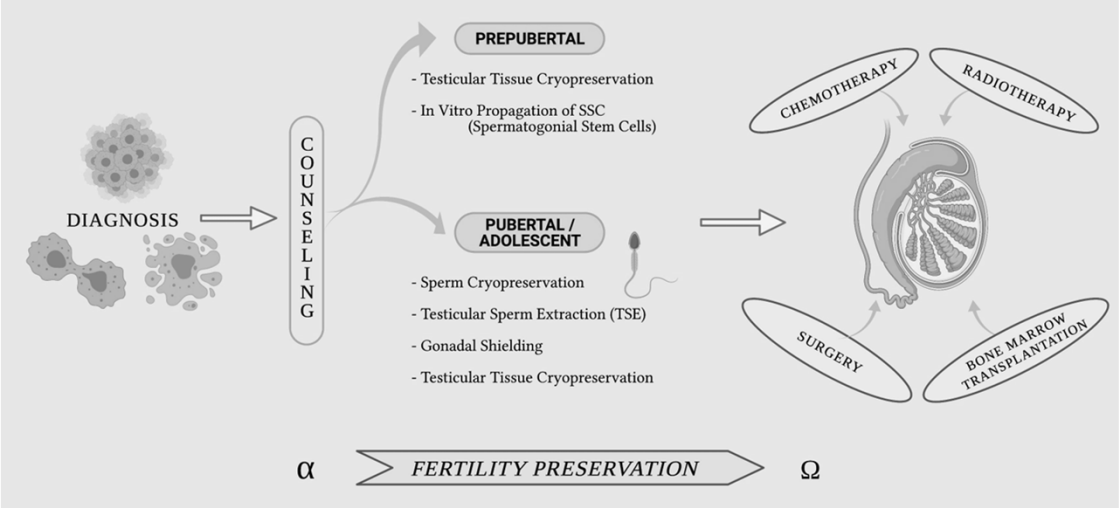
Pediatric and Adolescent Oncofertility in Male Patients

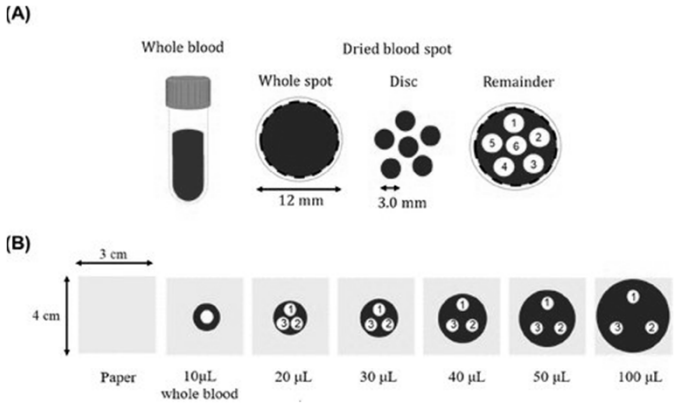


32

Numerous studies show the promise of AMH as a serum-based biomarker for the assessment of ovarian reserve in cancer patients and survivors.

It is important to note that assessment of ovarian reserve in certain cancer patients and survivors involves measuring very low AMH levels.

AMH assay that uses dry blood spots compared to the conventional serum or plasma samples. Dried blood spot specimens' stability makes it a practical alternative to venous blood. It opens new possibilities in AMH testing, such as comparison of historical to current patient results; simplified blood sampling for patients in remote locations or for those who are homebound.



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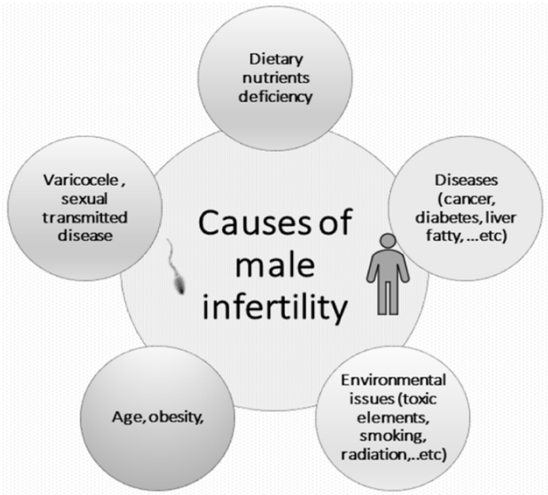
MALE INFERTILITY



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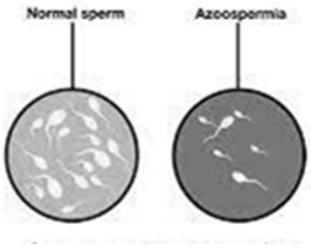
Biomarkers of common reproductive impairments in males

It is estimated that **up to 7% of men are affected** by infertility and 50% of fertility problems within a heterosexual couple are due to the man.

Several proteins expressed in the epididymis and testis were directly associated with fertility

SPACA = Sperm acrosome membrane-associated protein
TEX101 = Testis-expressed sequence 101 protein
ECM1 = Extracellular matrix protein 1

Total serum cathepsin D and K activity and total sialic acid as biomarkers for distinguishing between benign prostatic hyperplasia and prostate cancer.



obstructive azoospermia
non-obstructive azoospermia
pretesticular azoospermia

[ ] of seminal plasma proteins such as TEX101 and ECM1 can
be used as biomarkers for diagnosing azoospermia.

↓ regulation of several genes, such as SPATA3, SPACA4, FAM71F1, UBQLN3, GGN, and AKAP4 has been observed in infertile males.

↑ regulation of TMEM225, ADCY10, WBSCR28, GSG1, FSCN3, GTSF1L, and SPATS1 was identified in males with late maturation arrest.

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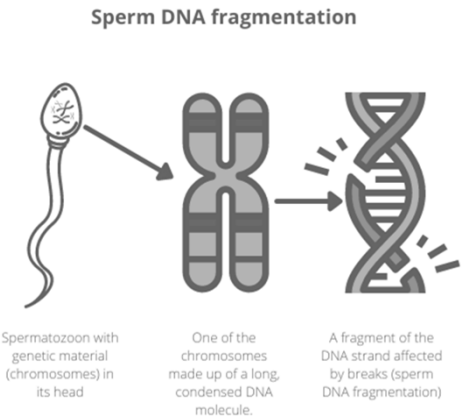
The DNA degradation index has been proven to be a noninvasive biomarker for identifying infertile individuals with varicocele, a condition that causes impaired testicular function and is associated with increased fragmentation in sperm DNA.

Seminal plasma can be used in the discovery of biomarkers because the seminal plasma proteome contains a plethora of proteins, including tissue-specific proteins, and may be used to monitor pathological processes



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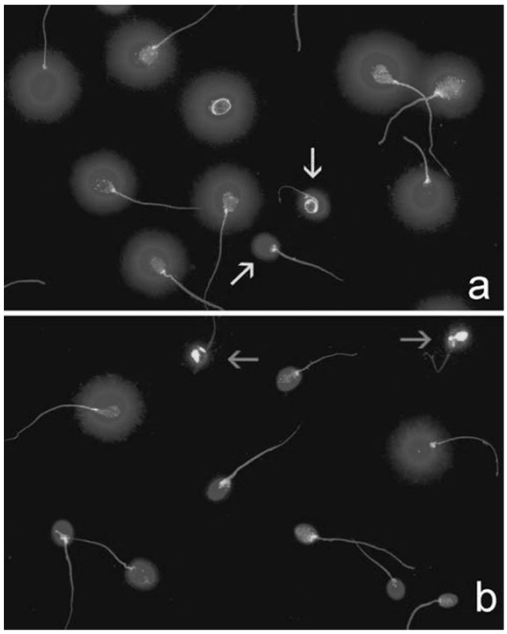

DNA Fragmentation Dynamics in Fresh Versus Frozen Thawed Plus Gradient-Isolated Human Spermatozoa

Sperm DNA fragmentation as visualized after application of the SCD test and staining using Gel Red. (a) Fresh sperm sample processed at baseline. Yellow arrows highlight sperm nuclei containing damaged DNA. (b) Fresh sperm sample after 24 h incubation. Green arrows show highly degraded sperm nuclei.

Gel Red staining provided high resolution images in the sperm chromatin dispersion (SCD) test.

•DOI: 10.3109/19396360903515430

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MALE INFERTILITY

Inhibins and Activins are gonadal hormones relevant for the assessment of testicular function.

Serum Inhibin B and Activin A levels have been correlated with several conditions affecting spermatogenesis and patients with idiopathic male infertility show higher levels of Activin A in serum and semen samples as compared to fertile donors.

Serum Inhibin B was also found to be useful in the prediction of azoospermia risk in testicular cancer patients' post-treatment.

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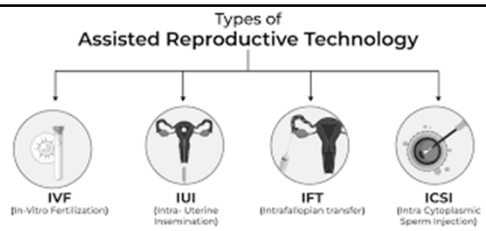
ASSISTED REPRODUCTIVE TECHNOLOGY



Prognostic role of AMH in assisted reproductive is well studied.

Numerous studies focusing on patients undergoing in vitro fertility and intracytoplasmic sperm injection (IVF/ICSI) report correlation between AMH and treatment outcomes. Seminal AMH levels were reported to be useful in predicting motile sperm recovery rate in cryopreserved semen from asthenozoospermic men. Seminal AMH may also be useful in selecting infertile patients for recombinant FSH treatment.

Though major focus has been on AMH, studies also show that serum Activin A can be a marker of outcomes in pregnancies conceived via IVF.

Levels of Inhibin B in follicular fluid correlates with presence of oocyte and Activin A/Inhibin B ratio in follicular fluid has also been shown to correlate with the number of oocytes retrieved.

Inhibin B levels were reported to gradually increase during controlled ovarian hyperstimulation procedure and correlated with outcomes.

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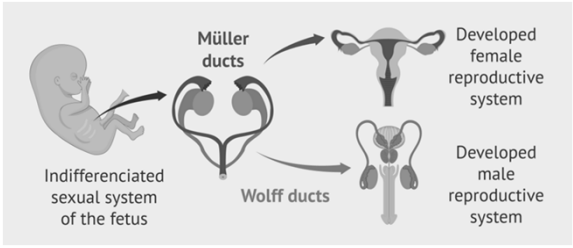
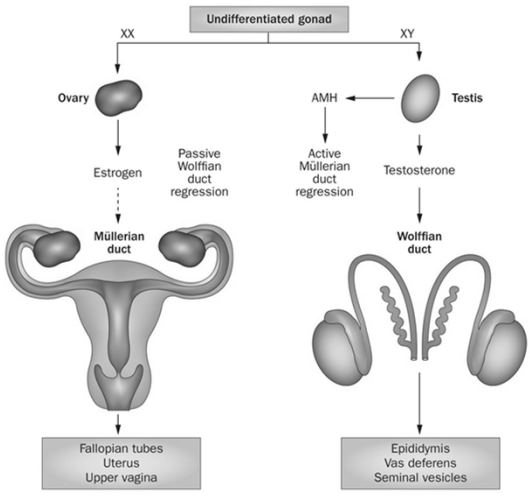
DISORDERS OF SEX DEVELOPMENT





During embryonic development, AMH plays an important role in sexual differentiation. In the male fetus the expression of AMH from primitive Sertoli cells causes Mullerian duct regression, while in the female fetus the absence of AMH allows for development of female reproductive organs. AMH levels at birth are very high in boys and very low to undetectable in girls. In cases of ambiguous genitalia, AMH measurement becomes essential to determine the presence of testicular tissue. Many other conditions involving disorders of sex development rely on AMH assessment for determining treatment options.

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Neohormones

Neohormones are a group of recently evolved hormones primarily associated to the success of mammalian development.
These hormones are specific to mammals and are not found in other vertebrates—this is because neohormones are evolved to enhance specific mammalian functions.

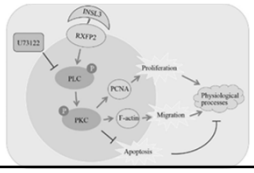
- important roles in regulating testicular descent (the testes descend into the scrotum during foetal development)
- preparing the sperm for internal fertilisation (the sperm fertilizes the egg within the female).

- essential for regulating early pregnancy,
- mammary gland development lactation (secretion of milk from the mammary gland),
- viviparity (allowing the fertilized egg to grow inside the female until they can exist independently).

Neohormones superimpose their actions on the hypothalamic-pituitary-gonadal axis and are not associated with other core bodily functions.

INSL3 (Insulin-like 3)

- anti-apoptotic in follicle selection (female)
- germ cell survival (male), excellent indicator of Leydig cell functional capacity, especially in aging males.



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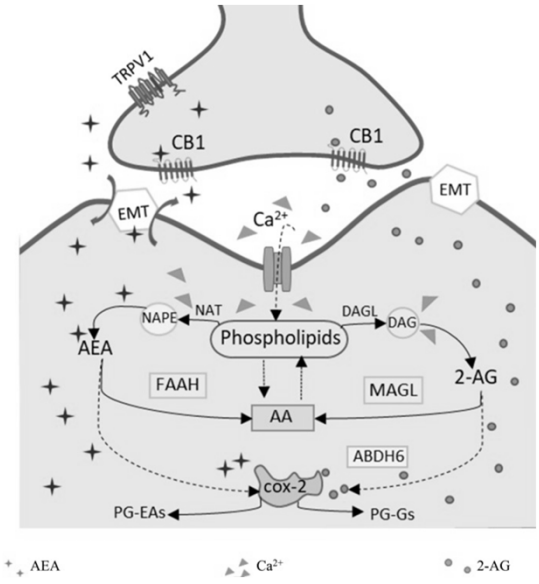
Endocannabinoids as potential biomarkers in reproductive medicine

Endocannabinoids (anandamide and 2-AG) have proven to be potential biomarkers of reproductive impairments.

They are a group of bioactive lipids that act as crucial signals in human reproduction.

Fluctuations in the balance between the degradation and synthesis of endocannabinoids lead to local changes in the human male and female reproductive tracts, which in turn adjust and control several pathophysiological processes, including sperm and oocyte maturation.



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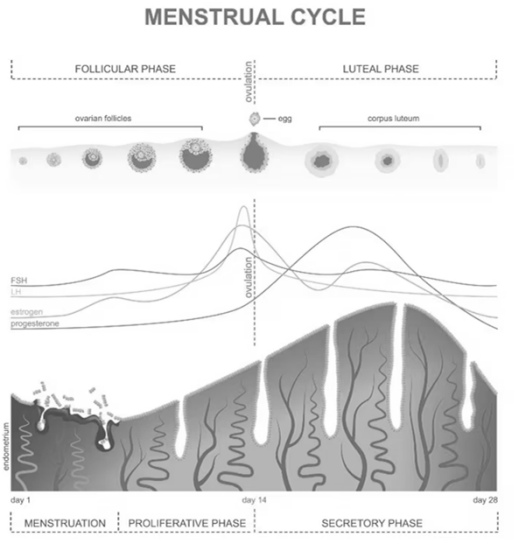

Biomarkers of an abnormal corpus luteum:

- Progesterone
- Inhibin
- Renin and Relaxin

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Biomarkers of the fallopian tube and pregnancy maintenance:

- Vascular endothelial growth factor (VEGF)

It is a dominant angiogenic factor important in vascular growth, remodeling, and permeability.
It regulates angiogenesis in the endometrium and corpus luteum.
VEGF also plays a key role in placentation and implantation.

Serum VEGF levels would be elevated in tubal ectopic pregnancy
Serum VEGF levels are usually elevated in patients with disseminated cancer.
The concentration of VEGF is elevated in endometriosis patients .
NGF-β and VEGF are principal neurotrophic factors of the male reproductive system.
NGF-β and VEGF levels = novel biomarkers of diabetes-induced testicular damage.

- Creatine kinase

It is an enzyme released in response to muscle damage, is used as a marker of fallopian tube damage and a biomarker for the diagnosis of myocardial infarction.

↑ level = ectopic pregnancies

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Biomarkers of abnormal fetal growth:

- Pregnancy-specific b-1-glycoprotein (Schwangerschaft protein 1, SP1)
- Human placental lactogen
- Human chorionic gonadotropin
- Pregnancy-associated plasma protein-A
- α-Fetoprotein

In complicated pregnancies:
SP1 ↓ with increasingly severe retarded intrauterine growth

hPL is secreted by the placenta.
It can be monitored during the first trimester of pregnancy.
hPL ↓ in pregnant women with tubal ectopic pregnancy

hCG is the only biomarker currently used routinely in clinical practice.
hCG is released by trophoblasts and an increase in its level indicates the viability and survival of an embryo. Circulating hCG could be used as a biomarker or a tool for monitoring oxidative stress during pregnancy.

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Biomarkers of normal implantation:

- Progestagen-associated endometrial protein/glycodelin (placental protein-14)
- Leukemia inhibitory factor

PP-14 regulates the uterine environment to make it suitable for pregnancy

It plays an important role in the occurrence and timing of the suitable sequence of events in the fertilization process.

In healthy women, serum PP-14 concentrations changes during the phases of the menstrual cycle

The concentration of PP-14 is lower in endometriosis-affected subjects

Leukemia inhibitory factor belongs to the interleukin-6 family of cytokines,

It is a key contributor to human reproduction, implantation, and inflammation.

It is involved in the regulation of differentiation and growth of embryonic stem cells, and primordial germ cells.

Leukemia inhibitory factor was associated with successful pregnancy initiation

It is a potential biomarker of pregnancy.

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Biomarkers for measuring the estrogenic effects of endocrine disruptors

Many environmental chemicals act as endocrine disruptors, such as dichlorodiphenyl-trichloroethane, dioxins, and polychlorinated biphenyls, which are anti-androgenic and estrogen-like in nature.

They impede natural hormonal action and cause infertility in males and females

Endocrine disruptors effects:

- Prostateand testicular cancers
- Hypospadias
- Undescended testis
- Abnormal sexual development
- Sertoli cell-only patterns
- Altered thyroid and pituitary gland

Biomarkers:
1. Complement component 3 and ornithine decarboxylase
2. Vitellogenin
3. pS2 and mucin 1
4. Progesterone receptor
5. Calbindin-D9k

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Table 2. Classification of biomarkers as neohormones, endocannabinoids, and biomarkers of reproductive impairments in males and females

Biomarker
Neohormones
· INSL3
· INSL4
· INSL5
· INSL6
Endocannabinoids
· Anandamide
Biomarkers of common reproductive impairments in males
· TMEM225, ADCY10, WBSCR28, GSG1, FSCN3, GTSF1L, SPATA3, SPACA4, FAM71F1, UBQLN3, GGN, AKAP4, TEX101, ECM1, cathepsin D, K activity, total sialic acid
· DNA degradation index, seminal plasma
Biomarkers of common reproductive impairments in females
· Biomarkers of an abnormal corpus luteum (progesterone, inhibin, renin, relaxin)
· Biomarkers of fallopian tube to maintain pregnancy (VEGF, creatine kinase)
· Biomarkers of abnormal fetal growth (SP1, hPL, hCG, PAPP-A, α-FP)
· Biomarkers of normal implantation (PP-14, LIF-14)
· Biomarkers of reproductive cancer (follistatin, CA-125, IL-8, IL-6)
· Biomarkers for measuring the estrogenicity of endocrine disruptors (VTG, complement C3, pS2, mucin 1, CaBP-9k, PR)

VEGF, vascular endothelial growth factor; SP, Schwangerschaft protein; hPL, human placental lactogen; hCG, human chorionic gonadotropin; PAPP-A, pregnancy-associated plasma protein-A; α-FP, alpha-fetoprotein; PP-14, placental protein-14; LIF, leukemia inhibitory factor; CA-125, cancer antigen 125; IL, interleukin; VTG, vitellogenin; C3, component 3; CaBP-9k, calbindin-D9k; PR, progesterone receptor.

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Biomarkers of reproductive health in wildlife.

Biomarker	Sample types	Information derived from biomarker	Measurement techniques	Limitations	Citations
Steroid Hormones					
Androgen metabolites	serum, plasma, urine, saliva, feces, hair, nails/horn, feathers, baleen	1) reproductive status - males 2) adrenal function	1) radioimmunoassay 2) enzyme immunoassay 3) high performance liquid chromotography 4) liquid chromatography tandem mass spectrometry 5) ultraconvergence chromatography tandem mass spectrometry	1) most useful if samples of serum/plasma, urine, feces, and saliva assessing steroids are collected 3–5 times per week from focal individuals which can be difficult to do in wildlife 2) steroid concentrations from hair/nails/horn/feathers/baleen can be difficult to interpret in the context of time without knowing how long the given sample was growing prior to collection	[18];[22];[42];[48–80];[214];[239–249];[473–494]
Estrogen metabolites	serum, plasma, urine, saliva, feces, hair, nails/horn, feathers, baleen	evidence for follicular maturation			
Glucocorticoids	serum, plasma, urine, saliva, feces, hair, nails/horn, feathers, baleen	adrenal response to stress			
Progesterone metabolites	serum, plasma, urine, saliva, feces, hair, nails/horn, feathers, baleen	1) evidence for ovulation and corpus luteum formation 2) estrous cycle length and characteristics 3) pregnancy diagnosis: felids, giraffe species			

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Protein Hormones					
Anti-Mullerian Hormone	serum, tissue samples	1) follicular reserve 2) testicular maturation	1) radioimmunoassay 2) enzyme immunoassay 3) liquid chromotography tandem mass spectrometry	can only be measured in serum, urine (LH/FSH), or tissue samples which limits utility in free ranging wildlife as these types of samples are hard to collect in such populations	[18];[22];[42];[81–194];[214];[229–238];[473–494]
Follicle Stimulating Hormone	serum, urine	1) role of pituitary in follicular growth 2) role of pituitary in Sertoli cell development and spermatogenesis			
Inhibin	serum, tissue samples	1) function of ovarian granulosa cells or FSH suppression required for normal follicular cycling in females 2) function of Sertoli cells and subsequent spermatogenesis in males			
Insulin	serum	elevations in insulin are correlated with infertility in captive housed wildlife			
Leptin	serum	1) increases during pregnancy in many species 2) directly correlated to body fat quantity as it is an adipokine			
Luteinizing hormone	serum, urine	1) surge in this hormone is correlated with evidence for ovulation 2) role of pituitary in Leydig cell secretion of testosterone			
Oxytocin	serum, urine, saliva	1) maternal and paternal behavior & bonding with offspring 2) negatively correlated with aggression in animal species that live in social groups 3) milk letdown			
Prolactin	serum	Species specific roles in reproduction: 1) rodents - luteotropic 2) felids/canids/elephants - luteotropic & pregnancy maintenance 3) African elephant - follicular development, but also linked to acyclicity (hyper or hypoprolactinemia 4) kangaroo - embryonic diapause 5) all species - milk production			
Relaxin	serum, urine	1) corpus luteum function: swine, rats 2) placental function: felids, canids, lagomorphs, equids 3) pregnancy diagnosis: felids, elephants			

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Biomarker	Sample types	Information derived from biomarker	Measurement techniques	Limitations	Citations
Miscellaneous Hormones					
Melatonin	serum, saliva	1) correlated with seasonal reproduction 2) light pollution drives down serum	1) radioimmunoassay 2) enzyme immunoassay	1) can only be measured in serum, feces (T3/T4, prostaglandin metabolites), saliva (melatonin)	[18];[22];[42];[196–228];
					(continued on next page)
Prostaglandins	serum	melatonin levels and interferes with reproduction 1) corpora lutea establishment 2) maintenance of pregnancy/ placental function 3) changes in concentrations related to onset of parturition 4) onset of spawning and quality of eggs	3) liquid chromotography tandem mass spectrometry	which limits utility in free ranging wildlife as these types of samples are hard to collect in such populations	[285–291]; [473–494]
	Triiodothyronine (T3) / thyroxine (T4)	serum, feces			
	1) basal metabolic rate 2) seasonal regulation of GnRH secretion				

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Biomarker	Sample types	Information derived from biomarker	Measurement techniques	Limitations	Citations
Emerging Biomarkers					
Acute Phase Proteins	serum, plasma	non-specific markers of inflammation including: -serum amyloid A (SAA) -C reactive protein -haptoglobin -ceruloplasmin	1) radioimmunoassay 2) enzyme immunoassay 3) fluorescent immunoassay 4) liquid chromotography tandem mass spectrometry	1) primarily measured in serum or plasma which limits utility in free ranging wildlife (although ceruloplasmin in urine) and saliva in domestic species (i.e. pigs) so potential for expanding options for wildlife	[18];[22];[42]; [292–433]; [473–530]; [550–558]
Cytokines	serum, plasma, tissue	1) pregnancy specific patterns 2) regulate folliculogenesis 3) control oocyte maturation 4) contribute to luteolysis 5) contribute to testis function & development	1) radioimmunoassay 2) enzyme immunoassay 3) fluorescent immunoassay 4) liquid chromotography tandem mass spectrometry 5) immunohistochemistry	1) primarily measured in serum, plasma or tissue which limits utility in free ranging wildlife. Urinary measures are possible, but would require validation 2) some methods of detection are costly	
Microbiome	feces, milk, semen, vaginal secretions, tissue	1) immune status 2) relative stress an animal is under at the microbial composition is correlated with glucocorticoid levels 3) for GI microbiome both related to estrogen in circulation and dietary estrogens	16 s sequencing	1) costly to perform sequencing 2) need to know how to perform bioinformatics analysis or have a collaborator that has this expertise	
microRNAs	serum, plasma, milk, urine, semen, tissue	1) related to fecundity 2) correlated with stage of estrous cycle 3) inflammation (i.e. endometritis) 4) pregnancy detection 5) correlated with spermatogenesis	1) qPCR 2) microarrays 3) RNA sequencing	1) costly to perform sequencing 2) need to know how to perform bioinformatics analysis or have a collaborator that has this expertise if running sequencing 3) for qPCR need to design primers which could be challenging because of differences in gene homology between domestic species and wildlife	
eDNA	soil, water	which organisms comprise the biodiversity of a specific ecosystem	qPCR coupled with DNA sequencing	1) costly to perform sequencing 2) to identify organisms in the ecosystem their genome has to have been previously sequenced and identified 3) cannot differentiate between current or historical ecosystem residents unless carbon dating techniques are coupled with the DNA sequencing	
Reactive oxygen species	serum, plasma, saliva, exhaled breath condensate, semen, IVF/IVM media, tissue	1) correlated to normal ovulation 2) made by a healthy corpus luteum 3) correlated with normal sperm capacitation and acrosome reaction 4) elevated levels in males correlated with sperm DNA damage	1) chemiluminescence or fluorescence 2) chromatography 3) fluorescent proteins 4) electron spin resonance. 5) spectrophotometry 6) flow cytometry or confocal microscopy with fluorescent dye DAF-FM	1) costly methods of detection 2) specialized equipment needed for detection 3) unique expertise needed for some of detection methods	

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Table 3.1 Comparative proteome profile of human semen associated with infertility and the identification of probable biomarkers of infertility

Condition	Sample	Methods used	Proteins identified	Reference
Azoospermia	Seminal plasma	2D-MALDI-TOF MS	ACPP and KLK3	Starita-Geribaldi et al. (2001)
Azoospermia	Seminal plasma	2D-MALDI-TOF MS/MS	CLU, AZGP1, PAEP, APCS, NPC2, CRISP1 and SOD1	Starita-Geribaldi et al. (2003)
Azoospermia	Seminal plasma	2D-DIGE-LC-MS/MS	STAB2, CP135, GNRP, PIP, NPC2	Yamakawa et al. (2007)
Azoospermia	Seminal plasma	Strong cation exchange chromatography-LTQ-Orbitrap MS/MS	COL6A2, GGT7, SORD, PGK2, LDHC, ZPBP2 and ELSPBP1	Batruch et al. (2012)
Azoospermia	Seminal plasma	LC-MS/MS	LDHC, SPAG11B, MUC15, TEX101 and CEL	Drabovich et al. (2011)
Azoospermia	Seminal plasma	2-DIGE-MALDI-TOF-TOF MS/MS and 2-DIGE-LC-LTQ-Orbitrap MS/MS	PAP	Davalieva et al. (2012)
Azoospermia	Seminal plasma	SRM	TEX101 and ECM1	Drabovich et al. (2013)
Azoospermia	Seminal plasma	ID-NanoLC-MS/MS	LGALS3BP	Freour et al. (2013)
Azoospermia	Seminal plasma	Immunocapture-SRM	TEX101	Korbakis et al. (2015)
Azoospermia	Spermatozoa	2D-MALDI-TOF/TOF MS	TEKT2 and TPI1	Zangbar et al. (2016)
Varicocele	Spermatozoa	2D-DIGE	HSPA5, SOD1, ATP5D	Hosseinifar et al. (2014)
Varicocele	Spermatozoa	ID-LC-LTQ-Orbitrap MS/MS	CRISP2 and ARG2	Agarwal et al. (2015b)

(continued)

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Table 3.1 (continued)

Condition	Sample	Methods used	Proteins identified	Reference
Varicocele	Spermatozoa	ID-LC-LTQ-Orbitrap MS/MS	GSTM3, SPANXB1, PARK7, PSMA8, DLD, SEMG1, and SEMG2	Agarwal et al. (2015c)
Varicocele	Spermatozoa	ID-LC-LTQ-Orbitrap MS/MS	TEKT3 and TCP11	Agarwal et al. (2016b)
Varicocele	Spermatozoa	ID-LC-LTQ-Orbitrap MS/MS	HSPA2, ODF2, CCT6B	Agarwal et al. (2016c)
Varicocele	Seminal plasma	NanoUPLC-ESI-MS^E	G3P, PARK7, SOD, S100-A9, MDH	Camargo et al. (2013)
Varicocele	Seminal plasma	2D-ESI-QTOF MS/MS	UPP1, SEMG1, SEMG2, PIP, CYTS	Zylbersztejn et al. (2013)
Varicocele	Seminal plasma	2D-ESI-QTOF MS/MS	ALBU, CLU, SEMG1, SEMG2 and PSMA6	Del Giudice et al. (2013)
Varicocele	Seminal plasma	NanoUPLC-ESI-Orbitrap MS/MS	CAB45 and CRISP3	Del Giudice et al. (2016)
Asthenozoospermia	Spermatozoa	2D-PAGE	COX6B, HIST1H2BA and HSPA2	Martinez-Heredia et al. (2008)
Asthenozoospermia	Spermatozoa	2D-Nano-HPLC-ESI-MS/MS	PTPN14	Chao et al. (2011)
Asthenozoospermia	Spermatozoa	NanoUPLC-MS^E	HSPs	Parte et al. (2012)
Asthenozoospermia	Spermatozoa	2D-MALDI-TOF-TOF MS	COX6B and HSPA2	Hashemitabar et al. (2015)
Asthenozoospermia	Spermatozoa	2D-MALDI-TOF MS	PATE1	Liu et al. (2015)
Asthenozoospermia	Seminal plasma	ID-LC-MS/MS	PARK7	Wang et al. (2009)
Globozoospermia	Spermatozoa	2D-MS	ZNF174 and CAPZA3	Luo et al. (2008)
Necrozoospermia	Spermatozoa	2D-DIGE-MALDI-TOF MS/MS	SPANXa/d, SAMP1 and ODF2	Liao et al. (2009)

(continued)

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Table 3.1 (continued)

Condition	Sample	Methods used	Proteins identified	Reference
Oligoasthenozoospermia	Seminal plasma	LC-LTP-Orbitrap MS/MS	TBCB, AACT and ALDR	Herwig et al. (2013)
Oligoasthenozoospermia	Seminal plasma	1D-LC-MS/MS	CST3, AZGP1, TIMP1, SEMG1, and KLK3	Sharma et al. (2013c)
Oligoasthenozoospermia	Seminal plasma	2D-NanoLC-ESI-Q-TOF MS	NPC2, LGALS3BP, LCN1 and PIP	Giacomini et al. (2015)
Unexplained infertility	Spermatozoa	NanoLC-LTQ-Orbitrap MS	SPATA 24, ROPN1L, CRISP2, HSPA2, HSPA5, HSPB1, STIP1, CLU	McReynolds (2014)
Semen oxidative stress	Spermatozoa	1D-LC-MS/MS	HIST1H2BA, MDH2, TGM4, GPX4, GLUL, HSP90B1, HSPA5	Sharma et al. (2013b)
Semen oxidative stress	Spermatozoa	1D-LC-MS/MS	CLGN, TPPII, DNAI2, EEA1, HSPA4L, SERPINA5	Ayaz et al. (2015)
Semen oxidative stress	Seminal plasma	1D-LC-MS/MS	PIP, SEMG2, ACPP, CLU, AZGP1, KLK3, CST4, ALB, LTF, FN1, MIF and LGALS3BP	Sharma et al. (2013a)
Semen oxidative stress	Seminal plasma	1D-LC-MS/MS	MME	Agarwal et al. (2015a)
Semen oxidative stress	Seminal plasma	Shotgun proteomic analysis	FN1, MIF, G3BP, MUC5B	Intasqui et al. (2015)
Sperm DNA fragmentation	Spermatozoa	NanoUPLC-ESI-MS^E	HIST1H2AH, LTF, ODF1 and SPACA4, ZPBP2	Intasqui et al. (2013a)
Sperm DNA fragmentation	Seminal plasma	NanoUPLC-ESI-MS^E	ALB, NPC2, PATE4 and EDDM3A	Intasqui et al. (2013b)
Sperm DNA fragmentation	Seminal plasma	NanoUPLC-ESI-Orbitrap MS/MS	PSMA5	Intasqui et al. (2016)

(continued)

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Table 3.1 (continued)

Condition	Sample	Methods used	Proteins identified	Reference
Sperm mitochondrial alterations	Seminal plasma	NanoUPLC-ESI-Orbitrap MS/MS	ANXA7	Intasqui et al. (2016)
Sperm acrosome defects	Seminal plasma	NanoUPLC-ESI-Orbitrap MS/MS	ERP44 and GSTM3	Intasqui et al. (2016)
Diabetes	Spermatozoa	2D-DIGE-MALDI-TOF MS/MS	PIP, ODF1 and SEMG1	Kriegel et al. (2009)
Diabetes	Spermatozoa	2D-DIGE-MALDI-TOF-TOF MS/MS	SEMG1, CLU, LTF and GLB1L	Paasch et al. (2011)
Failed fertilization	Spermatozoa	2D-NanoESI-Q-TOF MS/MS	ODF2	Pixton et al. (2004)
Spinal cord injury	Seminal plasma	NanoUPLC-ESI-MS^E and 2D-ESI-Q-TOF MS/MS	ACTCM, ACTBM, ACTGM, NRAP, ACTN3, SYNE1 and SPTA2	da Silva et al. (2013)
Spinal cord injury	Seminal plasma	Dimethyl labeling-strong anion exchange LC-MS/MS	SERPINA1, SERPINA5, A2 M, ACR, KLK2, KLK3, KLK11 and PRTN3	da Silva et al. (2016)
Epididymitis	Spermatozoa		ATP5B, TUBA1A and TUBB4B	Pilatz et al. (2014)
Androgen deficiency	Seminal plasma		AZGP1, ACPP and PIP	Milardi et al. (2014)
Smoking	Seminal plasma	2D-ESI-QTOF MS/MS	ZA2G, SODE, PTGDS, ANAX3, CALM and AIAT	Fariello et al. (2012)
Smoking	Seminal plasma	NanoUPLC-ESI-Orbitrap MS/MS	LCN2, ORM1PRELP, SCGB2A1 and CEL	Antoniassi et al. (2016)

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