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EFFECT OF *Moringa Oleifera* LEAVES ON PHYSIOLOGICAL RESPONSE, HORMONAL CHANGES AND SEMEN QUALITY OF MALE RABBITS UNDER NORTH SINAI CONDITIONS

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ABSTRACT*:* *This study was intended to evaluate the effect of Moringa Oleifera leaf meal (MOLM) on reproductive hormones and semen quality traits in male rabbits. A total number of 24 New Zealand White (NZW) rabbits at 42 weeks of age, having an average body weight of 3.00 ± 0.15 kg, were randomly divided into four equal treatment groups of six rabbits each. The 1st group (control) was fed a basal diet. The 2nd, 3rd and 4th treatments were fed on diets at 2.5, 5 and 7.5% MOLM inclusion levels of the total diet, respectively. Blood samples were collected through the marginal ear vein from each rabbit for biochemical and hormonal assay using standard procedures. In addition, semen samples were collected weekly for 8 weeks and were analysed for semen quality traits. The ejaculate volume, sperm concentration, live sperm, normal sperm, and sperm motility of the experimental group of male rabbits were all significantly ($P \leq 0.05$) higher than those of the control group. The results also showed a higher level of gonadotropic hormones (FSH, LH) and testosterone in rabbits fed on moringa compared to control group rabbits, a decrease in total cholesterol and LDL-cholesterol in the groups fed diets containing moringa. It was also noted that there was an increase in the levels of total protein and HDL-cholesterol in the same groups compared to the control group. Plasma ALT and AST decreased with all treatments of MOLM, which indicates that it has a role in improving liver health.*

In conclusion*, this study showed that the replacement of 2.5, 5 and 7.5% of MOLM inclusion levels in the total diet seems to have a positive effect on blood biochemical, physiological response, hormonal changes and semen quality in male rabbits, and it could be used as a sexual promoter.*

Keywords: *Moringa Oleifera, blood biochemical, sex hormone, Physiological response, semen quality.*

INTRODUCTION:

Fertility in animals depends on the quality of their sperm (Dalton, 2011). Infertility has become a dangerous problem, more than 90% of male infertility cases are linked to low sperm counts or poor quality of semen, or both (Ekere *et al.,* 2013). Mammalian reproductive physiology is primarily regulated by several hormones and the central nervous system. Temperature and nutrition can have a direct or indirect effect on hormones and the gland's activities, as well as the reproductive process (George *et al.,* 2017). Inappropriate environmental signals can lead to a drop in semen quality and fertility (Rasooli *et al.,* 2010). Heat stress is one of the most factors that lead to a disturbance in the fertility rates of males, this is evident from the decrease in the conception rate and litter size at birth after mating with males exposed to heat stress during the summer season vs winter season (Marai et al., 2008In addition, Agarwal *et al.* (2008) and Ahmad *et al.* (2012) indicated that elevated levels of free radicals and imbalances in the antioxidant-defence system result in the activation of the sympathetic nervous system because high ambient temperature activates the hypothalamic–pituitary–adrenal axis. As indicated, an increase in free radical accumulations is associated with decreased sperm motility, significant increases in defective sperm, and DNA defects that result in infertility (Potts *et al.,* 2000).

Therefore, oxidative stress has been linked to infertility. This indicates that approximately 80% of infertile animals may be due to high oxidative stress (Wu *et al.,* 2020). Since maintaining semen quality characteristics of rabbit bucks through the heat stress period is essential in hot areas. Thus, the protective effect of antioxidants may be essential to improve the semen quality traits of male rabbits in hot and semi-hot areas (Jimoh and Ewuola, 2018*;* Jimoh *et al., 2021)*

Medical plants have been used as an aphrodisiac to improve sexual performance, libido, and sperm quality (Prabsattroo *et al., 2012)* and treat infertility (Bhatia *et al., 2010;* Jimoh *et al.,* 2020) or as fertility-enhancing agents (Sumalatha *et al.,* 2010; Bhatia *et al.,* 2010), and improved testosterone level (Prabsattroo *et al.,* 2015).

Moringa Oleifera has been utilized as a source of plant antioxidants as one of the Moringaceae family's medicinal plants. It is rich in antioxidant vitamins, including vitamin C (Makkar and Becker 1996; Konmy *et al.,* 2016), vitamin E and β-carotene (Kidmose *et al.,* 2006), and polyphenols. These components make their antioxidant activity higher than traditional antioxidants like ascorbic acid (Yang *et al.,* 2006). In addition, the leaves of *Moringa Oleifera* contain simple sugar, rhamnose, carotenoids, phytates, phenolic acids, flavonoids (Amaglo et al., 2010 and Coppin *et al.,* 2013), alkaloids, isothiocyanates, and glucosinolates triterpenoid (Kidmose *et al*., 2006; Augustin *et al.,* 2011). Moreover, it includes vitamin A (Ferreira

et al., 2008), magnesium, iron, vitamin B1, and vitamin B2 (Makkar and Becker, 1996; Konmy *et al.,* 2016), as well as anti-inflammatory chemicals (Yang *et al.,* 2006).

Therefore, this study examined the effect of varied *Moringa Oleifera* leaves meal (MOLM) levels on reproductive hormones, biochemical blood parameters, and reproductive performance in male rabbits.

MATERIALS AND METHODS:

Experimental design:

The research was done throughout the summer on a private farm in El Arish, North Sinai Governorate, Egypt. 24 male New Zealand White (NZW) rabbits aged 6 months and averaging 3.00±0.15 kg in body weight were utilized. Randomly, rabbits were divided into four equal treatment groups. Individual galvanized wire cages measuring 60X55X40 cm were utilized to house the rabbits in a naturally ventilated facility. Batteries were accommodated with feeders for pelleted rations and automatic drinkers. Animals were kept under similar management conditions.

Throughout the experiment, ambient temperature and relative humidity were measured twice daily, at 6:00 am and 2:00 pm and the daily mean was calculated. The temperature–humidity index (THI) was calculated according to the method described by Marai *et al*. (2001):

$$THI = db°C - [(0.31 - 0.31\ (RH))\ (db°C - 14.4)]$$

Where:

- db°C = Temperature of the light bulb
- RH = Relative humidity percent/100

The resulting THI values were then categorised as follows: <27.8 = No heat stress, 27.8 - <28.9 = Moderate heat stress, 28.9 - <30.0 = Severe heat stress, and 30.0 and above = Extreme heat stress (Marai *et al.,* 2001).

Experimental diets:

Moringa (*Moringa oleifera)* was obtained from Agricultural Research Center in Dokki, Egypt, and was used in diets at the rate of 0, 2.5, 5 and 7.5 % as a replacement of the diets. *Moringa Oleifera* leaves are composed of 91.48% Dry matter, 26.5% crude protein (CP), 11% crude fibre (CF), 10.1% total ash,6.35% Either Extract (E.E), 3200 Kcal/Kg feed Digestible energy. Feed and clean water were provided daily ad libitum.

The light period was maintained at 16 hr light: 8 hr dark per day. The diets were designed to suit the NRC-recommended nutritional needs of rabbits (NRC, 1977). Table 1 displays the ingredient composition of the experimental diets.

Table (1): Composition and calculated analysis of the experimental diets.

Ingredients, %	*Moringa oleifera Leaves Meal %*			
	0	**2.5**	**5**	**7.5**
Yellow corn	9	9	9	8.5
Soybean meal, 44%	14.43	13.44	12.18	11.08
Wheat bran	28.57	27.06	27.76	26.92
Barley	15	15	13.06	13.00
Alfalfa hay	30	30	30	30
Limestone	1	1	1	1
Dicalcium Phosphate	1.2	1.2	1.2	1.2
Salt	0.5	0.5	0.5	0.5
Vit. + min. premix*	0.3	0.3	0.3	0.3
MOLM	0	2.5	5	7.5
Total	**100**	**100**	**100**	**100**
Calculated analysis(%)				
Crude protein	18	18	18	18
Digestible energy (DE)	2628.58	2636.39	2629.97	2631.23
Crude fibre	12.2	12.28	12.22	12.36
Either extract	3	2.9	2.9	2.8
Lysine	0.83	0.79	0.79	0.78
Methionine	0.3	0.3	0.3	0.3
Calcium	1.05	1.12	1.19	1.27
Phosphorus	0.5	0.55	0.61	0.64

* Each three kilogramme serving of vitamin-mineral premix includes the following quantities of vitamins and minerals: Vitamin A, 12,000,000; Vitamin D3, 3,000,000 IU; vitamin E, 700 mg; Vitamin K_3, 500 mg; vitamin B_1, 500 mg; Vitamin B_2, 200 mg; Vitamin B_6, 600 mg; Vitamin B_{12}, 15 mg; Folic acid, 10 mg; Choline chloride, 1000 mg; Niacin, 3000 mg; Biotin, 6 mg; Panathonic acid, 670 mg; manganese sulphate, 80 g; iron sulphate, 1 g; zinc sulphate, 70 g; Copper sulphate, 0.2 g; Iodine, 1 g; Cobalt sulphate, 300 mg; Selenium, 0.3 g.

Measurements:

Blood analysis:

At the end of the experiment, after two months, blood samples (5.0 ml) were withdrawn from marginal ear veins for each treatment in the morning before feeding. Samples were collected in test tubes with heparin to obtain plasma and in test tubes without heparin to obtain serum. Blood samples were centrifuged at 3000 rpm for 15 min, and samples were stored until analysis.

Using radioimmunoassay (RIA), the blood concentrations of FSH and LH were measured in duplicated samples. FSH/LH kits were obtained from Bio-code Company-Belgium and used according to the procedure included with each kit. FSH and LH hormone detection sensitivities per test tube

were 0.02 ng/ml and 0.14 ng/ml, respectively. Using enzyme-linked immune sorbent assay (ELISA) kits, testosterone hormone levels in the blood were determined (Diagnostics Test Canada, Inc., Ontario, Canada). The hormone detection sensitivity per test tube was 0.25 ng/ml.

Moreover, collected serum samples were subjected to biochemical analysis of each parameter according to the manufacturers' exact steps of its kit. Total protein was analyzed by Sonnenwirth and Jarett, (1980), albumin (Doumas, 1971), and total cholesterol were measured using the method of Stein (1986).

Semen collection:

Semen samples were collected once weekly for 8 weeks between 8.00 am and 9.00 am by means of an artificial vagina using a female teaser rabbit. A different artificial vagina was used for each collection.

The amount of semen in the collecting tube was read in milliliters. Each ejaculate was analyzed to determine the following physical sperm characteristics:

Live/dead sperm percentage: The live dead spermatozoa percentage was assessed using an eosin-nigrosin staining mixture ***(***Blom, 1959) by testing 100 sperm cells.

Abnormal sperm percentage: The percentage of abnormal spermatozoa was determined in a smear prepared for a live/dead sperm test.

Sperm cell concentration: A weak eosin solution (Smith and Mayer, 1955) was employed to determine the concentration of sperm cells. Microscopically, spermatozoa were counted using an upgraded Neubauer haemocytometer slide (GmbH and co., Brand stwiete 4, 200 Hamburg 11, Germany).

Total sperm output: An equation was used to measure it according to the method described by Hafez, (1985):

Total sperm output = Sperm concentration x Total volume of ejaculate ($x10^6$)

Sperm quality factor (SQF): Calculated according to the following pattern was used:

$$\textbf{SQF} = \frac{\text{sperm concentration} \times \text{Ejaculate volume} \times \text{live normal sperm}}{\mathbf{100}}$$

Statistical analysis:

Statistical analysis was performed using Analysis of Variance (ANOVA) and the General Linear Model (GLM) Procedure from the SAS User's Guide (SAS, 2004).

The significance of mean differences was determined using the multiple range test developed by Duncan (1955).

RESULTS AND DISCUSSION:

Temperature-humidity index (THI):

The temperature-humidity index (THI) calculated in Table (2) revealed that the rabbits were subjected to severe heat stress throughout the duration of the experiment.

Table (2): Mean values (±SEM) of ambient temperature (°C), relative humidity (%) and temperature–humidity index (THI) throughout the course of the experiment

Summer Months	Average temp. (0C)		Averages RH (%)		Averages (THI)	
	Min	Max	Min	Max	Min	Max
Mid-June	25.51 ±0.15	37.04 ±0.38	32.34 ±1.43	82.81 ±1.88	23.44	35.48
July	25.80 ±0.24	36.01 ±0.26	33.46 ±1.36	83.61 ±1.13	23.93	34.99
August	26.91 ±0.33	37.91 ±0.52	35.92 ±1.52	83.66 ±1.36	24.81	36.82
Mid-September	26.05 ±0.26	36.96 ±0.32	33.94 ±1.47	83.36 ±1.27	24.06	35.75
Average	26.07 ±0.18	36.98 ±0.31	33.91 ±1.07	83.36 ±0.98	24.06	35.76

Blood analysis:

Table (3) shows a significant (P≤0.05) increase in total protein, albumin, globulin and HDL-cholesterol for rabbits fed diets containing MOLM compared to the control group. On the contrary, the result showed that using MOLM in rabbit diets had a significant (P≤0.05) decrease in total cholesterol, LDL-cholesterol, ALT and AST compared to the control group. These results agree with Samar *et al.,* (2016), who showed a significant (P≤0.05) diminishing in total cholesterol and LDL. The same result was obtained by Idemudia *et al.,* (2013), who found that HDL levels increased in rats. On the same side, Ezzat *et al.,* (2014) *and* El-Speiy *et al.,* (2021) got the same results when they used oils and extracts of moringa in feeding rabbits. Also, Mehta *et al.,* (2003) showed decreased total cholesterol, triglyceride, VLDL, LDL-cholesterol, and an increase in the HDL-cholesterol when using moringa fruit. Also, Voemesse *et al.,* (2018) showed a significantly (P≤0.05) increase in the level of total protein and albumin when using *Moringa Oleifera* leaf in the chicken's diet.

Table (3): Effect of used *Moringa oleifera* leaves meal on some blood biochemical parameters (Mean ±S.E) of male rabbits

Traits	Control	*Moringa oleifera leave meal %*		
		2.5	**5**	**7.5**
T. protein(g/dl)	5.01d ±0.13	5.90c ±0.28	6.39b ±0.24	6.77a ±0.37
Albumin (A) (g/dl)	2.67d ±0.14	3.17c ±0.12	3.49b ±0.14	3.75a ±0.15
Globulin (G) (g/dl)	2.34c ±0.16	2.73b ±0.19	2.91a ±0.17	3.02a ±0.21
T. Cholesterol (mg/dl)	90.96a ±2.71	86.40b ±2.82	84.82bc ±2.40	82.69c ±2.55
HDL- Cholesterol (mg/dl)	39.01d ±1.42	49.74c ±1.45	53.54b ±1.35	54.92a ±2.22
LDL- Cholesterol (mg/dl)	50.83a ±2.68	35.59b ±1.69	30.23c ±1.49	26.75d ±1.48
ALT (U/L)	32.50a ±0.83	25.38b ±0.90	23.65c ±0.65	22.83d ±0.60
AST (U/L)	37.41a ±0.85	31.03b ±0.67	28.59c ±0.88	27.19d ±0.76

[a,b,c] Means in the same row with different superscripts are significantly different (P<0.05).

These results could be attributed to moringa being a rich source of protein, β-carotene, calcium, potassium, vitamin C and other active substances. These components work as a good source of natural antioxidants in addition to the presence of phenolics, flavonoids and carotenoids (Shahidi *et al.*, 1992). In addition, moringa may have a role in promoting cholesterol secretion in the digestive system.

On another side, the experiment's results showed that using moringa leaves affected liver function, where using moringa led to a significant decrease in ALT and AST activities. Moreover, moringa leaves increased total protein and albumin, reflecting this plant's ability to metabolise protein and stimulate the regeneration of hepatic tissue in rabbits, increasing protein synthesis in the liver and improving the functional status of liver cells. This shows the role it plays in maintaining the health and safety of liver tissues.

Serum FSH, LH and testosterone hormone measurements:

The results in Table (4) showed that using MOLM in rabbit diets had significant (P≤0.05) effects on LH, FSH and testosterone concentrations for males fed different MOLM diets compared with that in the control group. The results archived that the treatments in which moringa leaves were used

Table (4): Effect of used *Moringa oleifera* leaves meal of male rabbit on serum LH, FSH, and testosterone hormones (Mean ±S.E)

Traits (g)	Control	*Moringa oleifera leave meal %*		
		2.5	5	7.5
LH (ng/ml)	44.48d ±1.28	55.25c ±1.97	59.85b ±1.25	63.63a ±1.60
FSH (ng /ml)	47.28d ±1.44	57.47c ±2.22	63.53b ±1.41	69.38a ±1.36
Testosterone (ng/ml)	2.18c ±0.68	2.89b ±0.58	3.37a ±0.57	3.75a ±0.73

[a,b,c] Means in the same row with different superscripts are significantly different (P<0.05).

in feeding male rabbits achieved the highest rate in the LH, FSH and testosterone hormones in the blood compared to the control group. These results agree with Khalifa *et al*., (2016), who showed that *Moringa oleifera* increased LH, FSH and testosterone levels in the blood. On the same side, Gouda *et al.*, (2020) found that the use of moringa led to a significant (P≤0.05) increase in the proportion of the testosterone hormone in the blood of bucks rabbits.

These results may be because the moringa can affect the hypothalamus releasing hormone (GnRH), which stimulates the anterior pituitary to produce the gonadotropins hormones released into the blood and thus increase the level of LH, FSH and testosterone (Ekaluo *et al.,* 2013). In addition to the above, flavonoids, alkaloids and other phytochemical content are well known for increasing testosterone hormone concentration (Alabi *et al.*, 2017).

All of this affects sexual performance in male rabbits. This is related to an increase in FSH secretion since FSH plays a role in facilitating spermatogenesis (Ojeda and Skinner, 2006). In addition, increasing serum testosterone levels improve sexual behaviour and erection (Türk *et al*., 2008). This could be due to the bioactive component like flavonoids that may stimulate the testis or through a hypothalamus-pituitary-testis-axis (Türk *et al*., 2008; Jimoh *et al*., 2021).

Furthermore, the increase in these hormones may be because the MOLM possesses potent antioxidant properties due to its high contents of phenolic compounds and isothiocyanate (Verma *et al.*, 2009; Coppin *et al*., 2013 and Tumer *et al*., 2015). Additionally, bioactive content such as tocopherol, carotene and beta-sitosterol in Moringa (Rajanandh and Kavitha, 2010) might have affected hormone synthesis.

Semen characteristics:

The result in Table (5) showed that administration of MOLM in male rabbits significantly (P≤0.05) increased ejaculate volume, sperm concentration, sperm quality factor, and total live sperm in all experimental groups as compared with the control group. Also, abnormal sperms were significantly (P≤0.05) decreased in birds fed diets containing MOLM than in control. These results agree with those (El-Harairy *et al*., 2016; George *et al.*, 2017; Ojo and Abdurahman 2017; El-Desoky *et al.*, 2017; Ajuogu *et al.*, 2018 and Gouda *et al.*, 2020) were they found a significant (P≤0.05) increase and improvement of semen quality on male rabbits fed diets contain Moringa Oleifera compared with control groups. On the other side, Abu Ahemen and Ikpechukwu (2013) and Ezzat *et al.*, (2014) found that the use of *Moringa Oleifera* had no adverse effect on the sperm quality of rabbit bucks.

Table (5): Effect of used *Moringa oleifera* leaves meal of male rabbit on semen characteristics (Mean ±S.E)

Traits	**Control**	***Moringa oleifera leave meal %***		
		2.5	**5**	**7.5**
Semen Appearance	Milky	Milky	Milky	Milky
Semen Viscosity	Normal	Normal	Normal	Normal
Ejaculate volume (ml)	0.43d ±0.02	0.52c ±0.02	0.61b ±0.03	0.69a ±0.04
Sperm concentration (x10^6/ml)	181.7d ±5.55	245.3c ±7.91	267.6b ±8.26	295.2a ±8.41
Total live sperm (%)	71.8d ±1.36	79.5c ±1.23	83.6b ±1.28	88.7a ±1.94
Normal sperm (%)	83.3d ±1.40	90.6c ±1.17	91.8b ±2.20	94.2a ±2.16
Abnormal sperm (%)	16.7a ±0.91	9.4b ±0.67	8.2c ±0.70	5.8d ±0.56
Dead Sperm (%)	28.2a ±1.36	20.5b ±0.73	16.4c ±0.78	11.3d ±0.94
Sperm quality factor	65.1d ±2.85	115.5c ±3.88	149.8b ±3.30	191.9a ±4.56
Total sperm output (10^6/ejaculate)	78.1d ±1.93	127.5c ±2.04	163.2b ±2.29	203.7a ±2.60

[a,b,c] Means in the same row with different superscripts are significantly different (P<0.05).

This could be attributed to MOLM containing natural nutrients and active components such as protein, a simple sugar, rhamnose, carotenoids,

phytates, phenolic acids, flavonoids, magnesium, iron, vitamin A, vitamin B1 and vitamin B2 and anti-inflammatory compounds. In addition, This could be due to MOLM containing some active substances that work as an aphrodisiac to improve sexual performance, libido and sperm quality (Prabsattroo *et al.,* 2012) and treat infertility or as fertility-enhancing agents and improve testosterone levels (Prabsattroo *et al.,* 2015). Alternatively, it may be because moringa contains vitamin C, vitamin E, β-carotene and polyphenols and other substances that act as powerful natural antioxidants that reduce the levels of free radicals that lead to high oxidative stress that leads to a decrease in sperm motility and a noticeable increase in abnormal sperm and DNA defects leading to infertility (Potts *et al.,* 2000).

Conclusion:

It is possible to draw the conclusion that increasing the amount of Moringa oleifera leaves in the diet of male rabbits led to an improvement in the quality of their semen. In addition, blood constituents, especially serum FSH, LH, and testosterone hormone, and also helped maintain liver tissue's health and integrity.

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تأثير استخدام اوراق المورينجا أوليفيرا على الاستجابة الفسيولوجية والتغيرات الهرمونية والسائل المنوي لذكور الارانب تحت ظروف منطقة شمال سيناء

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تهدف هذه الدراسة إلى تقييم تأثير استخدام أوراق المورينجا أوليفيرا في علائق ذكور الارانب على تقييم السائل المنوي وبعض مكونات الدم البيوكيميائية. تم تقسيم عدد ٢٤ ذكر من الأرانب النيوزيلندية البيضاء بعمر ٦ أشهر في نفس متوسط وزن الجسم تقريباً بشكل عشوائي إلى أربع مجموعات متساوية. المجموعة الأولى تغذت على نظام غذائي أساسي لايحتوي على المورينجا وتم تغذية المجموعات الثانية والثالثة والرابعة على علائق تحتوي على اوراق المورينجا بمستويات ٢.٥ و ٥ و ٧.٥٪ من إجمالي العليقة على التوالي. جمعت عينات الدم من وريد الاذن لتقدير تركيز بعض الهرمونات وبعض مكونات الدم الاخرى. بالإضافة إلى ذلك ، تم جمع عينات من السائل المنوي مره اسبوعيا خلال ٨ اسابيع متتالية لتقييم تلك العينات. أظهرت نتائج هذه التجربة أن استخدام المورينجا في علائق الأرانب أدى إلى تحسن كبير في حجم القذفة ومستوى تركيز الحيوانات المنوية ومعدل الحيوانات المنوية الحية والحيوانات المنوية الشاذه ومعامل كفاءه الحيوان المنوي مقارنة بنتائج المجموعه الضابطه. كما أظهرت النتائج أيضًا ارتفاع معدل الهرمونات في الدم مثل (FSH) و (LH) والتستيرون وانخفاض في الكوليسترول الكلي والكوليسترول البروتين الدهني منخفض الكثافة في المجموعات التي تتغذى على علائق تحتوي على اوراق المورينجا. كما لوحظ أن هناك زيادة في مستويات البروتين الكلي وكوليسترول البروتين الدهني عالي الكثافة مقارنة بالمجموعة الضابطة. كما انخفض مستوى إنزيمات الكبد الـ (ALT) و (AST) في جميع المعاملات المغذاه على علائق محتوية على اوراق المورينجا وهذا يشير إلى أن له دورًا في تحسين صحة الكبد.
التوصيه: أظهرت هذه الدراسة أن استخدام اوراق المورينجا في علائق الارانب له تأثير إيجابي على الكيمياء الحيوية للدم ، والاستجابة الفسيولوجية ، والتغيرات الهرمونية وجوده السائل المنوي على ذكور الأرانب.

الكلمات المرشدة: المورينجا ، مكونات الدم ، الاستجابة الفسيولوجية ، الهرمونات الجنسية ، جوده السائل المنوي.