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Relationship of Seminal Plasma Anti-Müllerian Hormone Concentration with Sperm Morphology and Sperm DNA Damage

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What's known on the subject? and What does the study add?

Recent investigations have demonstrated that seminal plasma anti-Müllerian hormone (AMH) was associated with sperm count and motility. There was no relation between both semen and blood AMH levels and Kruger morphology and sperm DNA breaks. Observing high numbers of TUNEL-positive sperm in groups, including the normozoospermic group, points to the importance of detecting DNA breaks in idiopathic infertility cases. Both DNA fragmentation testing and conventional semen analysis can be used together for the evaluation of male fertility potential.

Abstract

Objective: This study examines the relationship between seminal plasma anti-Müllerian hormone (AMH) levels and sperm morphology and sperm DNA fragmentation.

Materials and Methods: Semen and blood samples were obtained from volunteers. There were four patient groups that are normozoospermia (n=46), oligoasthenoteratozoospermia (n=18), azoospermia (n=19) and teratozoospermia (n=68), based on semen analysis results. Serum follicle-stimulating hormone, luteinizing hormone, testosterone, serum and seminal plasma AMH levels were measured. DNA fragmentation of sperm was assessed by the TdT-mediated dUTP nick-end labeling (TUNEL) test.

Results: Azoospermic group showed the highest blood AMH levels. The seminal AMH level of normozoospermic patients was found to be significantly lower compared to the oligoasthenoteratozoospermia group. No significant associations between seminal AMH, sperm morphology, and sperm DNA damage were observed. No significant difference was observed among the groups regarding sperm DNA fragmentation.

Conclusion: A large number of TUNEL-positive cells in normozoospermic patients demonstrates that DNA damage of sperm may also occur in normal sperm parameters individuals. The measurement of serum and seminal AMH levels does not provide any additional benefit during the evaluation of male infertility.

Keywords: Sperm, seminal plasma, anti-müllerian hormone, TUNEL

Introduction

Infertility affects about a quarter of couples in the world (1). The male-originated factors lead to half of the cases (1). Additionally, 50% of the causes of male infertility are still not entirely determined (2). Low fertility can be detected in men, even with normal semen parameters (3). Even though sperm parameters are normal, 15.5% of men may have idiopathic infertility (4).

Anti-Müllerian hormone (AMH) is a routinely used test since it indicates ovarian oocyte reserve in women and has been proven to be associated with various diseases. AMH is thought to play a role in spermatogenesis in which there is a direct link to the AMH type 2 receptor, which shows both autocrine and

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paracrine properties. AMH has been advised as a distinct and indirect marker for Sertoli cell function and spermatogenesis, respectively (5). Several studies have determined an association between AMH and sperm motility in men (6,7). But Aksglaede et al. (5) showed that AMH was not an accurate marker for evaluating semen quality (5).

Sperm cells are part of the important elements that transfer their genetic material to future generations. Although sperm cells have normal morphology and motility, DNA damage could occur. The known cause of DNA damage in spermatozoa is unresolved DNA nicks resistant to resolution in the remodeling of chromatin formed during spermatogenesis. Researched factors that cause DNA damage to spermatozoa are; programmed cell death, which is for eliminating defective germ cells, poor or atypical chromatin packaging, and lastly, oxidative stress due to the overproduction of reactive oxygen species (ROS) by infections, advanced paternal age, scrotal heat stress, exposure to xenobiotics or radiation, and so on (8). The DNA damage of sperm is generally measured by the TdT-mediated dUTP nick-end labeling (TUNEL) method, single-cell gel electrophoresis (Comet assay) and sperm chromatin structure assay (9). TUNEL assay is an important test that is used to detect DNA damage induced by ROS and unsuccessful apoptosis (3). The breaking of sperm DNA strand occurs in each ejaculate, and ROS can also induce these breaks. A high rate of damaged DNA was determined in the infertile patient's spermatozoa's cells with the TUNEL method, and significant low pregnancy rates were found in these patients (10).

Although seminal plasma AMH and its relationship with sperm count and motility were examined in previous studiesto the best of our knowledge, the association of seminal AMH with sperm morphology has been investigated to the healthy men. Therefore, we studied the possible relationship between serum and seminal AMH with sperm parameters, sperm morphology, and sperm DNA damage in men.

Materials and Methods

Study Population

This cross-sectional study was conducted by the Pamukkale University Local Clinical Research Ethics Committee (approval number: 60116787-020/8324). Two hundred four volunteer patients attended the study, and their semen and blood samples were collected between 26.07.2018-12.07.2019. Semen samples were examined. According to the results of semen analysis, the patients were classified into four groups that were teratozoospermia (n=68), normozoospermia (n=46), azoospermia (n=19) and oligoasthenoteratozoospermia (n=18). Patients with a history of cryptorchidism, chemotherapy, and cancer treatment (n=5), oligoteratozoospermia (n=14), asthenoteratozoospermia (n=21), oligozoospermia (n=10) were excluded from the study (Figure 1).

Semen Analysis

After 3-5 days of sexual abstinence, semen samples were obtained from the masturbation method. According to WHO 2010 guidelines, semen volume, total sperm count, the percentage of spermatozoa with rapid progressive motility, vitality, and percentage of normal spermatozoa were analyzed, as indicated volume ≥1.5 mL, concentration ≥15 million/mL, total count ≥39 million, progressive motility ≥32%, viability ≥58%, and normal morphology >4% (11). After the incubation period, the semen sample that became suitable for counting was dropped in the sperm counting chamber (Makler, Israel), and the sperms were counted using a phase-contrast microscope (Labomed, CXL, USA) for 100 fields under 40X magnification. The Preparing Semen Smears were stained the Diff-Quik method for Kruger morphology The slides were counted using a light microscope at 100X magnification. In each slide, 400 sperm cells were inspected, and the amount of normal, head, midpiece, and tail abnormalities were analyzed (Figure 2).

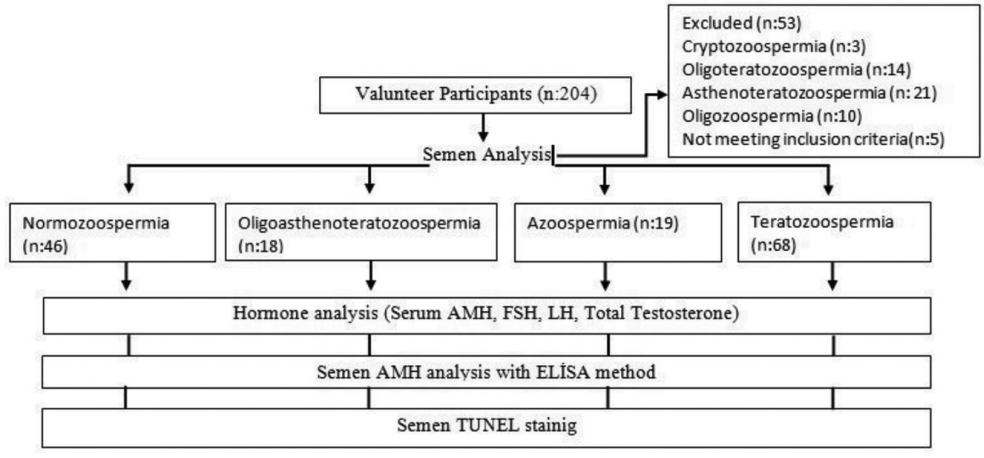

Figure 1. Flow chart of the overall experimental design

Hormone Analysis

Blood samples were collected between 8 and 10 am and centrifuged at 24 °C for 10 min at 1000 g. Semen samples were centrifuged at 1000 g for 20 min, and the seminal plasma was removed from the cell component. The total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and were interpreted in the Hormone Laboratory, Pamukkale University Hospital. Serum and semen AMH was measured using ELISA commercial kit (Elabscience, E-EL-HO317, USA) according to the manufacturer's instructions. Provided the micro ELISA plate in this kit has already been pre-coated with an antibody specific to Human AMH. Standards and samples were combined into appropriate micro ELISA plate wells and added with the specific antibody. Later incubated each microplate well was contained Avidin-Horseradish Peroxidase conjugate and biotinylated detection antibodies specific for Human AMH. The Substrate Reagent was added to each well, only those wells that contain Human AMH, biotinylated detection antibody. Adding stop solution was used to terminate the enzyme-substrate reaction. The spectrophotometry was employed todetermine the optical density at a wavelength of 450 nm.

TUNEL Assay

The TUNEL assay was used to determine apoptosis-related DNA strand breaks in spermatozoa, using the BIOTnA TUNEL Apoptosis Assay Kit (Biotna Biotech, 0160TA3081, TAIWAN). Briefly, one droplet of the liquefied sperm sample was air-dried onto a poly-lysine-coated glass slides, fixed with 4% paraformaldehyde. The cells were dehydrated by passing through an increasing alcohol series. The glass slides were stored at 4 °C. After storage, slides were rehydrated through a decreasing alcohol series. The cell slides were covered with sufficient drops of 3% Hydrogen Peroxide Block and were incubated for 5 min. The slides were first permeabilized with 0.2% Triton X-100 in PBS-Tween for 30 min. The slides were incubated with proteinase K for 15 min, then incubated in TdT Reaction Mixture for 1 h at 37 °C to allow DNA elongation. After stopping the enzyme-reaction, the DNA elongation was revealed by incubation of the cells with an anti-digoxigenin antibody coupled to peroxidase for 30 min. The peroxidase was revealed with diaminobenzidine. The sperm nuclei were counterstained with hematoxylin. slides were followed under a light microscope. On each slide, 200 cells were counted. Sperms with brown nuclei were evaluated as TUNEL positive. The apoptotic index was calculated by the following formula: Apoptotic index: (positive cells x 100)/total number of cells.

Statistical Analysis

Continuous variables are given as mean ± standard deviation, and categorical variables are represented as numbers and percentages. Shapiro-Wilk test was used for the data relevance to a normal distribution. When parametric test assumptions were provided one-way variance analysis was used; when parametric test assumptions were not provided, Kruskal-Wallis variance analysis was used to compare independent group differences. The relationships between the continuous variables were analyzed by Spearman correlation analysis, and the differences between categorical variables were examined with the chi-square test. $P<0.05$ value was evaluated as statistically significant.

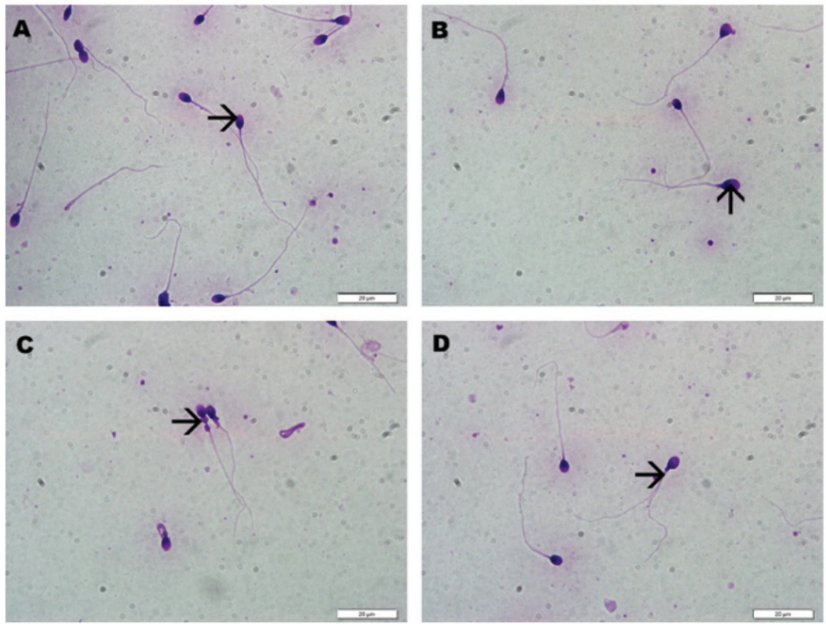

Figure 2. Morphological characteristics under light microscope A) Normal morfologia (arrow), B) Head anomaly (arrow), C) Midpiece Defect (arrow), D) Tail anomaly (arrow). Diff-Quick staining 100X

Results

Sperm Parameters

All groups of semen characteristics can be seen in Table 1. Semen volume was detected to be significantly lower in the azoospermia group than in the normozoospermic group (p=0.0001). The concentration was significantly higher in the normozoospermic group compared to other groups (p=0.0001). A statistically significant difference was observed between the total sperm count, total motile sperm count, total progressively motile sperm count (p=0.0001). Total motile sperm count and total progressively motile sperm count was statistically higher in the normozoospermic group than in the other groups. No significant difference was found between the normozoospermic, oligoasthenoteratozoospermic and teratozoospermic groups in the number of non-progressive motile sperm. The ratios of immobile sperm were significantly different between the groups. The rate of immobile sperm was significantly in the oligoastenoteratozoospermic group and is followed by the teratozoospermic group. When the normozoospermic group was compared with the other groups, the rate of immobile sperm was found to be significantly lower (p=0.0001). In the evaluation of Kruger morphology, the head anomaly was found to be the highest in the teratozoospermic group (90.68±5.13) and then in the oligoastenoteratozoospermic and normozoospermic groups (89.39±22.51), (82.1±6.37), respectively. A statistically significant difference was observed in the results (p=0.0001) (Table 2).

Hormone Analysis Results

FSH levels were significantly higher in the azoospermic group (18.55±17.5) U/L compared with the normozoospermic group (4.45±2.3) U/L (p=0.001) (Table 3). The FSH level of the oligoasthenoteratozoospermic group (8.51±6.07) U/L was detected as higher than the teratozoospermic group (4.42±3.78) U/L (Table 3). LH levels were significantly higher in the azoospermia group (8.51±6.07) U/L, compared to normozoospermic (5.19±2.06) U/L and teratozoospermic (5.47±2.43) U/L groups (Table 3). In the azoospermic group, a positive correlation was found between the high blood AMH level (955.1±1300.89) pg/mL and the blood FSH (8.51±6.07) U/L, (r=0.845, p=0.000) and LH (11.57±8.89) U/L, levels (r=0.701, p=0.001) (Table 3). Comparison of testosterone levels among the groups showed no significant difference (Table 3). The blood AMH level was highest in the azoospermia group (955.1±1300.89) pg/mL (Table 3). The study showed that the blood AMH level was lower in the teratozoospermic group (522.47±1003.22) pg/mL than the oligoteratoasthenozoospermic group (681.38±703.74) pg/mL (Table 3) (Figure 3).

Table 1. Comparison of semen parameters among four groups in study population (n=151)

	Normozoospermia		Oligoasthenoteratozoospermia		Azoospermia		Teratozoospermia		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	p
Semen volume (mL)	3.83±1.56	3.5 (1.5-10.2)	3.2±1.7	2.85 (1.4-7.4)	2.02±1.35	2 (001-4.5)	3.38±1.66	3 (0.7-9.7)	0.0001*
Sperm concentration (10^6/mL)	98.22±48.2	91.5 (24-265)	3.97±2.88	3.55 (0.1-12.5)	0±0	0 (0-0)	71.6±48.02	61.5 (0.01-230)	0.0001*
Total sperm count (10^6)	343.74±156.35	294.5 (84-840)	11.18±7.49	11.4 (0.37-30)	0±0	0 (0-0)	221.75±160.94	183.5 (0.02-722)	0.0001*
Total motile (%)	71.26±11.02	73 (46-91)	33.06±9.25	37 (14-43)	0±0	0 (0-0)	66.24±10.61	65 (45-88)	0.0001*
Sperm count (10^6)	238.59±106.54	217 (54-609)	3.69±2.53	3.75 (0.05-8.8)	0±0	0 (0-0)	150.96±123.15	120 (22-554)	0.0001*
Total progressively motile sperm count (10^6)	210.59±106.72	186 (42-546)	2.18±1.39	2.05 (0.03-4.8)	0±0	0 (0-0)	128.95±111.7	102 (18-504)	0.0001*
Progressively motile (%)	61.74±12.29	62 (34-86)	20.11±6.9	21 (8-31)	0±0	0 (0-0)	54.13±12.26	53 (32-80)	0.0001*
Non-progressively motile (%)	11.11±8.95	10 (3-64)	12.89±4.48	13.5 (4-22)	0±0	0 (0-0)	12.09±4.95	11 (4-26)	0.0001*

SD: Standard deviation, significance level*: p-value <0.05

There was also a significant difference in seminal AMH (pg/mL) levels between the groups (p=0.043) (Figure 4). Among the groups, normozoospermic patientswith seminal AMH levels were significantly lower than the oligoasthenoteratozoospermic group. There was no correlation between the blood and seminal AMH levels of the groups (p>0.05) (Table 3). In azoospermic patients, it was found that as the sperm volume increased, the semen AMH level also increased (r=0.658, p=0.006).

Analysis of DNA Damage

TUNEL results were similar among the groups. (Table 3) (Figures 5, 6). While there was a negative correlation (r=-0.337, p=0.016) between the head anomaly rate of Kruger morphology and the presence of TUNEL-negative cells in teratozoospermic patients, a positive correlation was found between the neck and tail anomaly (r=0.323, p=0.021, r=0.297, p=0.035, respectively) (Figure 5).

Discussion

In this study, there was no relationship between both semen and blood AMH levels and Kruger morphology and sperm DNA breaks. The presence of TUNEL-positive cells in all groups indicates the existence of DNA breaks.

Andersen et al. (7) found that individuals have very different AMH levels in seminal plasma from each other, and they suggested that seminal plasma AMH might be used as a marker for sperm production, but the predictive value gap is limited (7). Serum AMH levels are useful clinic parameters for infants and children in predicting testicular function (12). Because AMH is present in just mature Sertoli cells, it may be a marker for semen quality (7). It was reported that subfertile men have low levels of AMH compared with normal men (13-15). However, other studies didn't show consistent data for this association (16,17). Aksglaede et al. (5) showed that AMH concentration

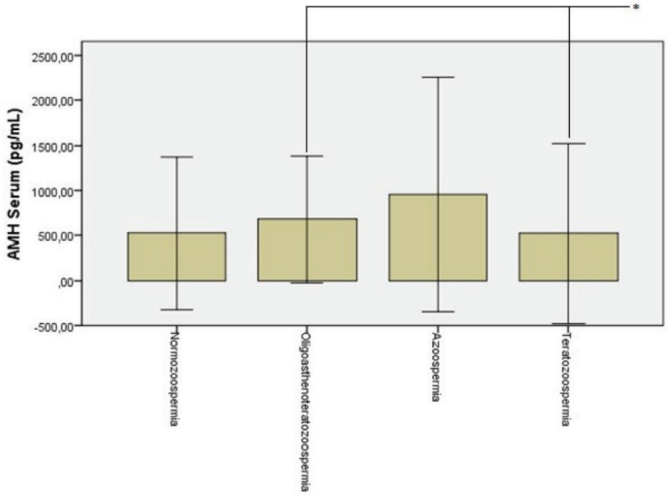

Figure 3. Comparison of blood AMH values between groups. Blood AMH levels of teratozoospermia patients were significantly lower than oligoastenothetospermia group. Significance level*: p<0.05

AMH: Anti-Müllerian hormone

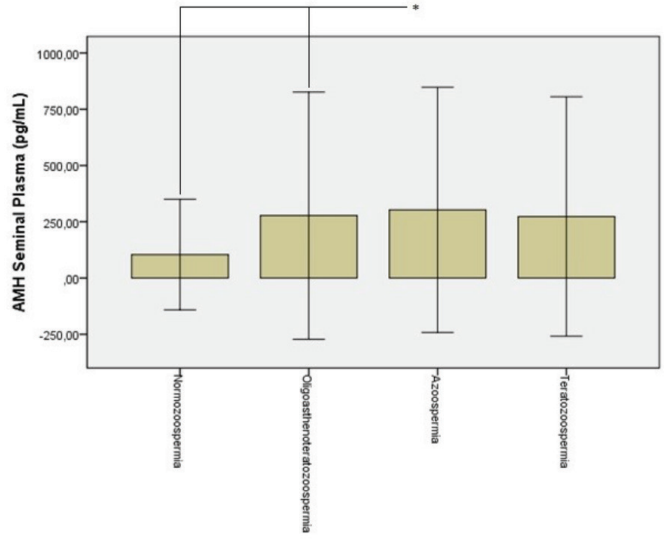

Figure 4. Comparison of semen AMH values between groups. Semen AMH levels of normozoospermic patients were significantly lower than oligoasthenoteratozoospermia group. Significance level*: p<0.05

AMH: Anti-Müllerian hormone

Table 2. Comparison of normal, head, neck and tail anomalies according to Kruger criteria between groups

	Normozoospermia		Oligoasthenoteratozoospermia		Azoospermia		Teratozoospermia		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	p
Normal morfologia (%)	4.63±0.83	4 (4-7)	0.76±0.83	1 (0-3)	0±0	0 (0-0)	2.16±1.05	3 (0-3)	0.0001*
Head anomalies (%)	82.15±6.37	82 (58-92)	89.39±22.51	96 (0-98)	0±0	0 (0-0)	90.68±5.13	92 (72-100)	0.0001*
Midpiece defects (%)	8.3±3.27	8.5 (3-20)	2.76±1.68	2 (1-6)	0±0	0 (0-0)	4.5±2.49	4 (0-15)	0.0001*
Tail defects (%)	4.93±3.84	4 (1-25)	1.82±1.13	1 (1-4)	0±0	0 (0-0)	2.71±2.93	2 (0-16)	0.0001*

SD: Standard deviation, significance level*: p-value <0.05

Table 3. Comparison of FSH, LH, testosterone, AMH and TUNEL values between groups

	Normozoospermia		Oligoasthenoteratozoospermia		Azoospermia		Teratozoospermia		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	p
FSH (U/L)	4.45±2.3	4.08 (0-11.02)	8.51±6.07	6.63 (1.28-25.57)	18.55±17.5	14.6 (0.42-62.5)	4.42±3.78	3.69 (0.86-28.83)	0.0001*
LH (U/L)	5.19±2.06	5.3 (0-11.65)	6.48±4.33	6.29 (0.1-19.14)	11.57±8.98	10.08 (0.69-34.39)	5.47±2.43	5.16 (0.1-17.12)	0.001*
Total testosterone (ug/L)	4.1±1.72	4.19 (0-8.49)	4.08±2.07	3.92 (0.1-8.94)	3.3±1.74	3.35 (0.11-8.22)	4.53±1.8	4.2 (1.24-10.31)	0.072
AMH serum (pg/mL)	525.15±849.85	236.8 (24.45-3552)	681.38±703.74	457.3 (0.58-2743)	955.1±1300.89	487.25 (8.44-4879)	522.47±1003.22	126.6 (1.46-4402)	0.018*
AMH seminal plasma (pg/mL)	104.11±245.65	36.11 (0-1522)	277.13±549.45	79.27 (0-2027)	302.76±544.92	57.68 (11.38-2075)	273.07±532.12	50.55 (7.15-2209)	0.043*
TUNEL (AI %)	26.26±21.87	21 (1-94)	22.29±16.84	18.5 (2-66)	-	-	18.98±17.82	12 (1-68)	0.163

SD: Standard deviation, significance level*: p-value <0.05

in serum could not associate the concentration of sperm, healthy males sperm motility, total sperm count, and they argued that the WHO criteria are still the gold standard method for evaluating semen quality (5). In our study, the azoospermic group showed the highest blood AMH levels. When the groups were compared within themselves, blood AMH highs were found undoubtedly lower in the teratozoospermic group in contrast to the oligotretoasthenozoospermic group. Considering these data, we consider that blood AMH checking in male patients may be misleading for evaluating infertility.

Small-scale studies showed a positive correlation between the AHM of seminal plasma and the concentration of sperm (6,18,19), but this correlation was not supported by other studies (13-20). Actually, opposite results were shown in terms of seminal AMH and the motility of sperm (19,20).

It appears that apoptosis in Sertoli and germ cells is regulated differently in the testis. While FSH deprivation-elevated DNA fragmentation without any effect on caspase activity, it had no impact on Sertoli cells (21).

Against these results, FSH levels in the oligoasthenoteratozoospermic group were significantly higher than those in the teratozoospermic group in the present study. LH levels were significantly higher in the azoospermia group compared to normozoospermic and teratozoospermic groups. The testosterone retraction in the testis is a reason for caspase activity and DNA fragmentation in Sertoli cells; however, it does not show any effect in germ cells (21). Related to this information, we found no significant difference between groups for testosterone levels in our study.

Duvilla et al. (22) showed that the seminal AMH mean value was calculated as 97.08 (±135.15) pmol/L in patients with normal parameters, 62.02 (±93.33) pmol/L in oligozoospermia patients, 13.12 (±31.94) pmol/L (n=67) in azoospermia patients (22). Kang-sheng et al. (23) studies focused on comparing of the AMH level of serum and seminal via FSH, LH, testosterone, and prolactin serum levels in infertile and fertile male groups. A positive

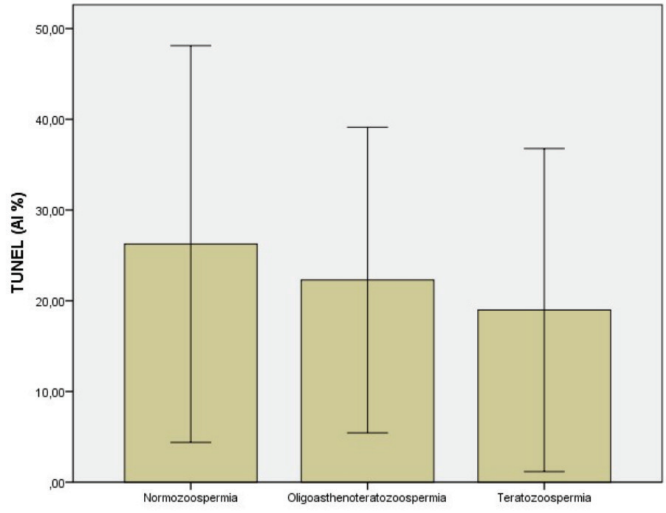

Figure 5. Comparison of sperm DNA fragmentation in normozoospermia (n=46), Oligoasthenoteratozoospermia (n=18) and teratozoospermia (n=68). Significance level*: p<0.05

correlation was shown the AMH level with sperm motility, class A sperm ratio, and sperm viability ($p<0.05$); however, it was not correlated with the sexual hormone level ($p>0.05$) (23). Contrary to this study, seminal plasma AMH concentration was very low in normozoospermic patients. We found that semen AMH levels were significantly lower in the normozoospermic patients than the oligoasthenoteratozoospermic group. We determined that as the sperm parameters improved, semen AMH levels decreased.

AMH promotes early-stage sperm maturation. Spermatogenesis is a consecutive process of cell maturation and differentiation. This hormonedependent process is regulated by a hypothalamic-pituitary-gonadal axis. Hormones in this process are the mixture of gonadotropin (GnRH) released from the hypothalamus, FSH and LH secreted from the pituitary gland, and T secreted by interstitial cells. Instead of directly interacting with spermatogenetic cells, FSH and T first bind to receptors on Sertoli cells, then feed the spermatogenic cells through a paracrine system. AMH secreted by Sertoli cells is seen in seminal plasma, but rarely passes through the blood-testis barrier. Therefore, the level of AMH in seminal plasma is much higher than that in serum. According to Kang-sheng et al. (23) the level of seminal AMH was higher than the level of serum AMH ($p<0.01$), and they announced that seminal AMH and serum AMH has not correlated ($r=0.026$, $p>0.05$) (23). Our study showed that there was a lower concentration of serum AMH levels compared with semen AMH levels. A correlation could not show blood and semen AMH levels in any group in accordance with that of the Kang-sheng et al. (23) ($p>0.05$).

To analyze the integrity of sperm DNA, a more objective marker of sperm function is recommended, unlike standard sperm parameters such as sperm motility (24,25). The high DNA fragmentation that we found in our study, even in normozoospermic patients, proves this recommendation. While our research shows a negative equation ($r=-0.337$, $p=0.016$) between the head anomaly rate of Kruger morphology and the presence of TUNEL-positive cells in teratozoospermic patients, a positive correlation was found between the neck and tail anomaly rates. These data showed us that DNA damage is inversely related to sperm morphology.

In a study conducted on 40 asthenozoospermic and 40 normozoospermic patients, sperm samples were analyzed. TUNEL-positive and motile sperm counts showed an inverse correlation (26). Irvine et al. (27) showed that ROS could trigger the breaking of sperm DNA strands, and this happens in each ejaculate. Henkel et al. (10) examined DNA fragmentation of semen samples taken from the IVF program. A significant correlation was found between the motile sperm count and TUNEL staining. Furthermore, they observed a low pregnancy rate in patients with high DNA fragmentation. Although, a direct correlation could not determine among the TUNEL-positive spermatozoa percentage and pregnancy or fertilization rate, patients with a high percentage of TUNEL-positive spermatozoa were found to have a lower pregnancy rate compared to patients with a low percentage (10). Unlike these studies, a significant difference between the groups regarding DNA fragmentation was not observed in our study. Saleh et al. (28) investigated and compared the DNA fragmentation indices of patients with normal and abnormal sperm parameters. They found that the index of DNA fragmentation was significantly surplus in infertile men who had normal sperm parameters (28). TUNEL positivity was found to be high in normozoospermic patients in our study; thus, our research also supports this study.

Study Limitations

Serum and semen AMH levels are affected by BMI. The limitation of our study is that the BMI values of the patients cannot be determined.

Conclusion

In conclusion, we did not find the possible relationship between blood and seminal AMH levels and sperm parameters.

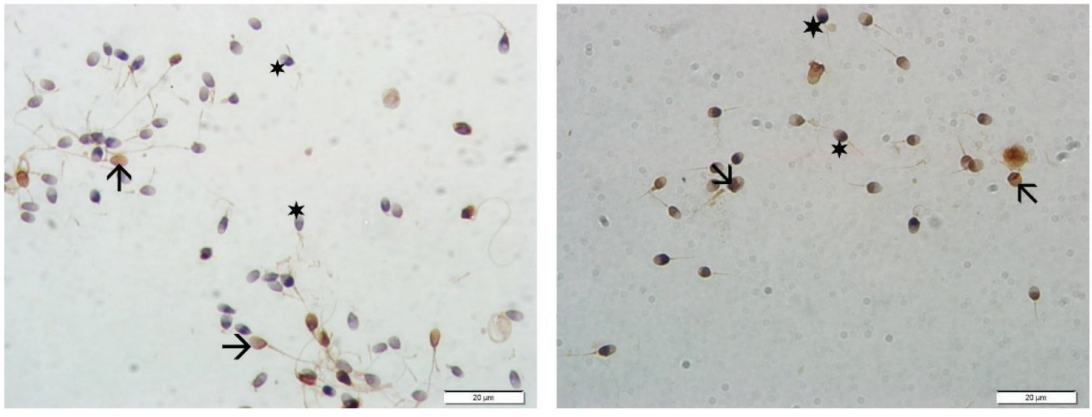

Figure 6. TUNEL staining, as TUNEL-positive (arrow), and TUNEL-negative cells (*), 100X

TUNEL-positive cells were observed in all groups, and the highest number was detected in the normozoospermic group. Because of the limited number of cases between groups, it is important to confirm these results with higher case numbers in future studies. Observing high numbers of TUNEL-positive sperm in groups, including the normozoospermic group, points to the importance of detecting DNA breaks in idiopathic infertility cases. Consequently, it can be considered that DNA fragmentation testing and conventional semen analysis can be used for evaluating male fertility potential.

Ethics

Ethics Committee Approval: This cross-sectional study was conducted by the Pamukkale University Local Clinical Research Ethics Committee (approval number: 60116787-020/8324, date: 01.02.2018).

Informed Consent: Informed consent was obtained from all the patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ç., T.T., İ.V.F., Concept: N.Ç., T.T., G.A.M., Design: G.T., N.Ç., C.K., Data Collection or Processing: G.T., N.Ç., C.K., Analysis or Interpretation: N.Ç., C.K., İ.V.F., G.A.M., Literature Search: G.T., N.Ç., C.K., Writing: G.T., N.Ç., C.K., T.T., İ.V.F., G.A.M.

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References

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;26:13-37.
2. Singh A, Agarwal A. The Role of Sperm Chromatin Integrity and DNA Damage on Male Infertility. The Open Reproductive science journal 2011;3:65-71.
3. Takeda K, Uchiyama K, Kinukawa M, Tagami T, Kaneda M, Watanabe S. Evaluation of sperm DNA damage in bulls by TUNEL assay as a parameter of semen quality. J Reprod Dev 2015;61:185-190.
4. Venkatesh S, Shamsi MB, Deka D, Saxena V, Kumar R, Dada R. Clinical implications of oxidative stress & sperm DNA damage in normozoospermic infertile men. Indian J Med Res 2011;134:396-398.
5. Aksglaede L, Olesen IA, Carlsen E, Petersen JH, Juul A, Jørgensen N. Serum concentration of anti-Müllerian hormone is not associated with semen quality. Andrology 2018;6:286-292.
6. Fujisawa M, Yamasaki T, Okada H, Kamidono S. The significance of Anti Mullerian Hormone concentration in seminal plasma for spermatogenesis. Hum Reprod 2002;4:968-970.
7. Andersen JM, Herning H, Witczak O, Haugen TB. Anti Müllerian hormane in seminal plasma and serum: association with sperm count and sperm motility. Hum Reprod 2016;31:1662-1667.
8. Esbert M, Pacheco A, Soares SR, Amorós D, Florensa M, Ballesteros A, Meseguer M. High sperm DNA fragmentation delays human embryo kinetics when oocytes from young and healthy donors are microinjected. Andrology 2018;6:697-706.
9. Ribas-Maynou J, García-Peiró A, Fernández-Encinas A, Abad C, Amengual MJ, Prada E, Navarro J, Benet J. Comprehensive analysis of sperm DNA fragmentation by five different assays: TUNEL assay, SCSA, SCD test and alkaline and neutral Comet assay. Andrology 2013;1:715-722.
10. Henkel R, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, Menkveld R, Gips H, Schill WB, Kruger TF Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil Steril 2004;81:965-972.
11. World Health Organization. World Health Organization Laboratory Manual for the Evaluation and Processing of Human Semen, 5th edn. Cambridge University Press, Cambridge, UK; 2010.
12. Lee MM, Donahoe PK, Silverman BL, Hasegawa T, Hasegawa Y, Gustafson ML, Chang YC, MacLaughlin DT. Measurements of serum Mullerian inhibiting substance in the evaluation of children with nonpalpable gonads. N Engl J Med 1997;21:1480-1486.
13. Al-Qahtani A, Muttukrishna S, Appasamy M, Johns J, Cranfield M, Visser JA, Themmen AP, Groome NP. Development of a sensitive enzyme immunoassay for anti-Müllerian hormone and the evaluation of potential clinical applications in males and females. Clin Endocrinol (Oxf) 2005;3:267-273.
14. Muttukrishna S, Yussoff H, Naidu M, Barua J, Arambage K, Suharjono H, Sathanandan M. Serum anti-Müllerian hormone and inhibin B in disorders of spermatogenesis. Fertil Steril 2007;2:516-518.
15. Goulis DG, Iliadou PK, Tsametis C, Gerou S, Tarlatzis BC, Bontis IN, Papadimas I. Serum Anti Mullerian hormone levels differentiate control from subfertile men but not men with different causes of subfertility. Gynecol Endocrinol 2008;24:158-160.
16. Tuttelmann F, Dykstra N, Themmen AP, Visser JA, Nieschlag E, Simoni M. Anti-Müllerian hormone in men with normal and reduced sperm concentration and men with maldescended testes. Fertil Steril 2009;5:1812-1819.
17. El-Halawaty S, Azab H, Said T, Bedaiwy M, Amer M, Kamal M, Al-Inany H. Assessment of male serum anti-Mullerian hormone as a marker of spermatogenesis and ICSI outcome. Gynecol Endocrinol 2011;27:401-405.
18. Fenichel P, Rey R, Poggioli S, Donzeau M, Chevallier D, Pointis G. Anti-Müllerian hormone as a seminal marker for spermatogenesis in non-obstructive azoospermia. Hum Reprod 1999;8:2020-2024.
19. Mostafa T, Amer MK, Abdel-Malak G, Nsser TA, Zohdy W, Ashour S, El-Gayar D, Awad HH. Seminal plasma anti-Müllerian hormone level correlates with semen parameters but does not predict success of testicular sperm extraction (TESE). Asian J Androl 2007;2:265-270.
20. Fallat ME, Siow Y, Belker AM, Boyd JK, Yoffe S, MacLaughlin DT. The presence of müllerian inhibiting substance in human seminal plasma. Hum Reprod 1996;10:2165-2169.
21. Tesarik J, Martinez F, Rienzi L, Iacobelli M, Ubaldi F, Mendoza C, Greco E. In-vitro effects of FSH and testosterone withdrawal on caspase activation and DNA fragmentation in different cell types of human seminiferous epithelium. Hum Reprod 2002;17:1811-1819.
22. Duvilla E, Lejeune H, Trombert-Paviot B, Gentil-Perret A, Tostain J, Levy R. Significance of inhibin B and anti-Müllerian hormone in seminal plasma: a preliminary study. Fertil Steril 2008;89:444-448.
23. Kang-sheng L, Hui Y, Xiao-dong M, Xiao-yong T, Ya-jun C. Application of Anti Müllerian Hormone in Diagnosis of Male Infertility. J Int Transl Med 2017;5:19-22.
24. Zini A, Bielecki R, Phang D, Zenzes MT. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 2001;75:674-677.

25. Evenson DP, Larson KL, Jost LK. Sperm chromatin struction assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002;23:25-43.

26. Moradi MN, Karimi J, Khodadadi I, Amiri I, Karami M, Saidijam M, Vatannejad A, Tavilani H. Evaluation ofthe p53 and Thioredoxin reductase in sperm from asthenozoospermic males in comparison to normozoospermic males. Free Radic Biol Med 2018;2:123-128.

27. Irvine DS, Twigg JP, Gordon EL, Fulton N, Milne PA, Aitken RJ. DNA integrity in human spermatozoa: relationships with semen quality. J Androl 2000;21:33-44.

28. Saleh RA, Agarwal A, Nelson DR, Nada EA, El-Tonsy MH, Alvarez JG, Thomas AJ Jr, Sharma RK. Increased sperm nuclear DNA damage in normozoospermic infertile men: a prospective study. Fertil and Steril 2002;78:313-318.