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Assessment of Biotin (Vitamin H) Supplementation to Sperm Preparation Medium on Outcomes of Intracytoplasmic Sperm Injection (ICSI)

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A R T I C L E I N F O

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A B S T R A C T

Improving sperm motility is essential for infertility treatment and the efficacy of assisted reproductive technology (ART). This study examines the effects of biotin in sperm wash medium on motility and early embryonic development. Semen samples from 150 human male patients aged 21-45, with low motility (Asthenozoospermia and Necrozoospermia), were divided into three groups: pentoxifylline (PTX), SpermMobil and biotin (study group). The optimal biotin ratio was determined through comet assay analysis and evaluated using computer-aided sperm analysis (CASA) before and after treatment. All groups underwent intracytoplasmic sperm injection (ICSI) to compare fertilization, cleavage, and pregnancy rates. A biotin ratio of 30:100 micromolar (μM) was found to enhance sperm DNA integrity and significantly improve motility and velocity. While biotin, PTX, and SpermMobil all enhanced motility, the biotin group exhibited better fertilization and cleavage rates, as well as higher pregnancy rates. Our findings suggest that incorporating biotin in sperm wash medium at a 30:100 ratio notably improves reproductive outcomes compared to PTX and SpermMobil.

1. Introduction

Infertility, as defined by the World Health Organization (WHO) [1], is a disorder of the male or female reproductive system characterized by the failure to conceive following 12 months of consistent unprotected sexual intercourse. WHO estimates indicate that 37% cases suffering infertility are attributed to female factors, meanwhile approximately 35% result from issues affecting both males and females [2]. This global health concern affects about 186 million individuals; one out of every six people suffers from infertility at some points in their lives. There are two types of infertility: primary infertility, which is the inability to conceive at all, and secondary infertility, which is the inability to conception after a prior successful pregnancy [3].

Male infertility is defined as a man's failure to conceive with a fertile partner after one year of unprotected sexual activity [4]. Up to 40% of infertility cases are categorized as idiopathic, where male factors account for 51% of these instances [5]. Various reasons contribute to male infertility, including both reversible and irreversible disorders [6]. Factors such as age, drug use, surgical history, environmental toxins, genetic issues, and systemic disorders may impact fertility in both partners [7]. The main causes of male infertility are low sperm counts (azoospermia/oligospermia), poor motility, and abnormal morphology [8].

Asthenozoospermia refers to decreased sperm motility, while necrozoospermia, also known as necrospermia, is characterized by a high percentage of non-motile or dead spermatozoa in a semen sample; both conditions significantly hinder the chances of achieving fertilization due to the presence of numerous non-viable sperm cells, even if the semen contains living sperm; their immobility prevents them from travelling from the vagina to the site of fertilization, thus impeding conception [9]. According to the WHO [10] recommendations, asthenozoospermic sperm samples should demonstrate total motility of less than 40%and progressive mobility, of less than 30%. Acute asthenozoospermia is additionally defined by complete

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sperm immobility or unusually low motility in the semen sample.

Several factors contribute to asthenospermia, as environmental toxins, infections, oxidative stress, inflammation, and genetic mutations [11]. To enhance the chances of conception in cases of reduced sperm motility, intracytoplasmic sperm injection (ICSI) is recommended. Initially introduced with in vitro fertilization (IVF), ICSI allows couples with severe male infertility to include this treatment option in their reproductive plans [12]. ICSI is an assisted reproductive technology (ART) where a single sperm is directly injected into an egg's cytoplasm. It has been proven effective when other ART methods fail to address severe male factor infertility [13].

In certain scenarios, spermatozoa extracted from testicular biopsies may lack motility. To enhance sperm motility, a chemical medium is employed to facilitate the injection of active spermatozoa for successful fertilization [14]. Chemicals such as pentoxifylline and SpermMobil have been utilized for this purpose; however, our research will focus on using biotin, a natural antioxidant.

Biotin (vitamin H) is a water-soluble B7 vitamin that is required for several metabolic processes, including fat, carbohydrate, and protein synthesis. It serves an important role in sustaining human health, growth, and development by performing crucial cellular metabolic activities [15]. Mammals have four carboxylases that need biotin as a cofactor to mediate fatty acid synthesis, branched-chain amino acid metabolism, and gluconeogenesis [16]. Notably, the motility and lifespan of human spermatozoa that have been cryopreserved are improved by biotin [17]. So, the aim of our research is to identify the optimal biotin dose for enhancing sperm motility and to compare it with conventional substances (pentoxifylline and SpermMobil).

2. Materials and methods

The study's participants were recruited from July 2023 to May 2024. All subjects who undergo the ICSI cycle. Semen samples were collected from 150 men with low motility (Asthenozoospermia and Necrozoospermia) subdivided to 3 groups: Biotin group (study group), pentoxifylline (PTX) group and SpermMobil group. Biotin (≥99%, Sigma-Aldrich, USA, Cat. No. B4639), Pentoxifylline (Sigma-Aldrich, USA, Cat. No. P1784), and SpermMobil (Fertipro NV, Belgium, Cat. No. 18001) were used in this study. All media and buffers were purchased from Life Global, Europe.

The consulting medical team of the Fertility Clinic's assisted reproduction unit at the International Islamic Centre for Population Studies and Research, Al Azhar University in Cairo, Egypt, conducted a clinical examination and evaluation of them. The subjects were between the ages of 21 to 45. After receiving complete information, each participant granted their consent. Each subject gave their informed consent. The Supreme Committee for Scientific Research Ethics at Fayoum University provided a formal ethical clearance letter (FU-SCSRE). The proposal's code number is EC 23170-a.

2.1. *Inclusion criteria*

Female Age ranges between 25 and 35 years old. No medical diseases, male and female (e.g. liver and kidney diseases). Severe male factors like Asthenozoospermia or Necrozoospermia.

2.2. *Technical approach of male subjects*

We measured the effect of biotin on improving sperm motility, so determining the appropriate biotin-to-semen ratio was crucial. Therefore, we tested three different ratios: equal ratios of biotin and semen (100:100), a ratio of 30 micromolar (μM) of biotin to 100 μM of semen (30:100), and finally a ratio of 5 μM of biotin to 100 μM of semen (5:100). To choose the ideal ratio, we performed a comet assay analysis. Statistical analysis of comet parameters (tail DNA %, tail length, and tail moment) was conducted using one-way ANOVA followed by Tukey's post hoc test. Tail moment values (mean ± SD) were 1.45 ± 0.32, 1.10 ± 0.27, and 1.26 ± 0.30 for ratios 100:100, 30:100, and 5:100, respectively (*$p < 0.05$*).

2.2.1. Assessing sperm DNA damage with the Comet assay

The alkaline comet assay, as explained by Simon [18], used to evaluate sperm DNA strand breakage. Also there is a need to prove that biotin can increase sperm parameters especially progressive motility so, Computer-aided sperm analysis (CASA) analysis is required to compare the samples of semen before and after adding biotin. CASA systems can identify and examine motile cells; they are perfect for examining the kinematics of spermatozoa [19]. The clinical examination and seminal fluid analysis were conducted according to the recommendations of the WHO [19].

2.2.2. Sperm preparation technique

All specimens were subjected to sperm preparation before ICSI [19]. To remove seminal plasma, thoroughly mix the semen sample and dilute it 1:1 with enriched media. Fill centrifuge tubes with the diluted suspension, capping each at 3 ml. After 5–10 minutes of centrifuging at 300–500g, gently dispose of the supernatants. Using careful pipetting, resuspend the sperm pellets in 1 milliliter of enriched media. Discard the supernatant after centrifuging once more for three to five minutes at 300 to 500 g. The sperm pellet should next be resuspended in the proper volume of enhanced media. Three groups of 150 samples are randomly selected, and each group is given a different washing media. Group 1: Semen samples washing with pentoxifylline (PTX): 50mM PTX (P1784, Sigma) with 50mMof pellet, and incubated for sixty minute at 37 °C before ICSI. Group 2: Semen samples washing with SpermMobil media 50mM SpermMobil with 50Mm of pellet, and incubated for sixty minute at 37 °C before ICSI. Group 3: Semen samples washing with biotin supplement, 100 mM of pellet containing 30 mM biotin (14400, Sigma) and incubated for sixty minute at 37 °C before ICSI. To assess sperm motility, a 10 μL solution was placed on a slide and examined under a microscope at various time intervals [19].

2.3. *Technical approach of female subjects*

2.3.1. Stimulation protocols and oocyte retrieval

Cases treated with an appropriate superovulation program (long or short protocol) to obtain enough eggs. ICSI was performed on retrieved oocytes

36 hours after hCG injection. The patient arrived in the operating room fasting, and the procedure was performed under general anaesthesia [20].

2.3.2. Ovum pick up:

Following separation using a Zeiss Stemi 2000-C stereo microscope, the OCC complexes were washed in global total w/HEPES Buffer (Life Global, Europe), and then they were cleaned and put into four well-filled plates with the same media [21].

2.3.3. Denudation:

Before being moved to a 100 µl drop of global total w/HEPES buffer (Life Global, Europe), the oocyte was held for 30 to 45 seconds in a 100 µl drop of buffered solution containing 80 IU/ml hyaluronidase (Life Global, Europe). We used a sterile stripper pipette to carefully aspirate the corona cells. The oocyte was cleaned in global total w/HEPES buffer after denudation, and all of the denuded oocytes were put in ICSI injection dishes with 10 µl microdrops of global total w/HEPES buffer and covered with 3 ml of sterile equilibrated mineral oil [21].

2.4. *CSI procedure*

After semen analysis and sperm preparation, samples were incubated until injection. ICSI was performed with clinicians blinded to treatment groups. One morphologically normal spermatozoon was transferred to each oocyte and mechanically trapped in polyvinylpyrolidone (PVP). Sperm for ICSI were analyzed and assessed. The injection process used an injecting needle and a holding pipette in a sterile dish. A 10 µl drop of global total w/HEPES buffer was introduced to a mature oocyte at 37 ºC, coated with mineral oil, and then sperm were added to another 10 µl drop of the same buffer. Van Steirteghem's approach [22] was followed to choose the best sperm, immobilize them in the PVP drop, and execute ICSI.

2.4.1. Embryo transfer and pregnancy assessment

The American Society of Reproduction's criteria were followed while transferring embryos from day three or day five to recipient subjects. Extra embryos of superior grade were cryopreserved. Serum-HCG levels were measured as a chemical pregnancy indicator 14 days after the transfer (positive if 20 IU/L or greater), and a transvaginal ultrasound was conducted to check for a clinical pregnancy (visible intrauterine gestational sac) after 6-7 weeks of amenorrhea [23].

2.5. *Statistical analysis*

SPSS software (version 26.0) was used to analyze the data. Normality was evaluated using the Shapiro-Wilk test. The three groups (Biotin, PTX, and SpermMobil) were compared using Tukey's post-hoc test and one-way ANOVA. Continuous data are displayed as mean ± standard deviation (SD). The same samples were compared before and after biotin treatment using a paired t-test. Additionally, the Chi-square (χ^2) test was used to compare categorical data, such as pregnancy rates, which are displayed as percentages (n, %). P-values less than 0.05 were regarded as statistically significant.

3. Results

We measured the role of biotin on improving sperm mobility, so it was necessary to determine the appropriate ratio of biotin to semen. Therefore, we tested three different ratios: equal ratios of biotin and semen (100:100), a ratio of 30 µM of biotin to 100 µM of semen (30:100), and finally a ratio of 5 µM of biotin to 100 µM of semen (5:100).

3.1. *Sperm DNA Integrity (Comet assay).*

To choose the ideal ratio, we performed a comet assay analysis and the result was that the ratio (30:100) is the ideal ratio, as it showed the lowest percent in the DNA damage, which is 2.3%, compared to the other ratios, as equal ratios showed DNA damage percent 3.4% and the third ratio (5:100) the percent of DNA damage was 2.8% in comparison to sample did not treated with biotin which it's percent of DNA damage was 5.4%. Accordingly the percentage of damage is highly significant at ($P \leq 0.05$) in control group than all groups treated with biotin this reported that biotin has beneficial effect on the sperm DNA integrity and the finest ratio is (30:100) as illustrated in Figures (1 & 2). Statistical analysis of comet parameters (tail DNA %, tail length, and tail moment) was conducted using one-way ANOVA followed by Tukey's post hoc test. Tail moment values (mean ± SD) were 1.45 ± 0.32, 1.10 ± 0.27, and 1.26 ± 0.30 for ratios 100:100, 30:100, and 5:100, respectively (*$p < 0.05$*).

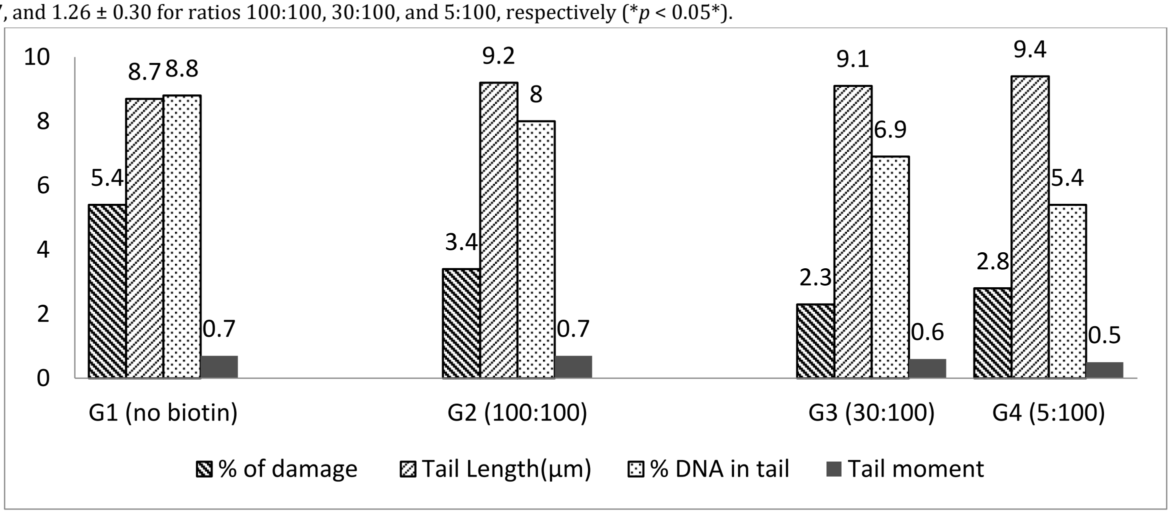

Fig. 1. Comet assay of Sperm DNA to assess the damage among different ratios of biotin to semen.

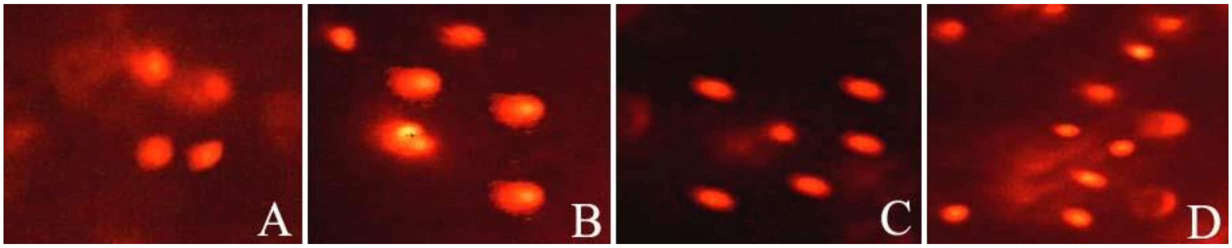

Fig. 2. Photomicrograph show Sperm comet assay DNA to assess the damage among different ratios of biotin to semen: (A) semen without biotin, (B) the biotin to semen in ratio (100:100), (C) the biotin to semen in ratio (30:100) and (D) the biotin to semen in ratio (5:100).

3.2. *CASA analysis before and after biotin:*

Also there is a need to prove that biotin has an ability to increase sperm parameters especially progressive motility so, CASA analysis is required to compare the samples of semen before and after adding biotin. The progressive motility is significantly increased from 18.58±4.87 to 41.39±10.21 after addition of biotin. Accordingly, the total motility increased from 49.28± 14.08 to 62.76± 13.59 after biotin. Similarity velocity parameters also significantly increased since VCL (Curvilinear Velocity) increased from11.02 ± 2.61 to 18.99 ± 4.22, VSL (Linear Velocity) increased from5.3 ± 1.52 to 10.25 ± 3.49 and VAP (Average Path Velocity) increased from 6.68 ± 1.57 to 12.97 ± 3.44 as illustrated in Table 1.

Table 1: The comparison between CASA analysis before and after biotin addition

CASA Analysis	progresive motility	non progresive motility	total motility	VCL(μm/S)	MAD(∘)	LIN(%)
Before biotin	18.58 ± 4.87	30.7 ± 12.72	49.28 ± 14.08	11.02 ± 2.61	25.18 ± 11.78	36.18 ± 11.05
After biotin	41.39 ± 10.21	20.77 ± 11.61	62.76 ± 13.59	18.99 ± 4.22	26.29 ± 7.23	55.86 ± 13.21
S. P value	5.265E-09*	0.028*	0.010*	4.17428E-07*	0.752	7.77707E-05*
	VSL(μm/S)	ALH(μm)	WOB(%)	VAB(μm/S)	BCF(Hz)	STR(%)
Before biotin	5.3 ± 1.52	3.37 ± 1.69	50.05 ± 14.56	6.68 ± 1.57	2.9 ± 1.27	63.28 ± 9.86
After biotin	10.25 ± 3.49	3.23 ± 0.97	70.73 ± 8.92	12.97 ± 3.44	3.36 ± 4.25	70.45 ± 19.31
S. P value	1.33630E-05*	0.612	3.61180E-05*	2.28505E-07*	0.679	0.196

(*) $p < 0.05$ represents significant differences between groups within the same column. S.; Significant, Yhe represented data are means± S.

3.3. *Comparing between biotin and another traditional ways to increase sperm motility:*

150 men patients participated in this investigation. All participants undergo ICSI cycle. All male with Asthenozoospermia are divided into 3 groups: Group 1: Semen samples treated with pentoxifylline (PTX) before ICSI (50 cases). Group 2: Semen samples treated with SpermMobil media before ICSI (50 cases). Group 3: Semen samples treated with biotin media before ICSI (50 cases).

3.3.1. Sperm Physical Characteristics.

The basic semen parameters as sperm concentration, total motility and morphology (sperm abnormality), were assessed for determining the difference between; pentoxifylline (group 1), SpermMobil (group2) and biotin (group 3) as shown in Table 2.

Table 2: Comparison between sperm parameters among studied male groups (n=150)

Sperm parameters	Group 1 (50)	Group 2 (50)	Group 3 (50)	Significance
Sperm count×10^6/mL	6.60 ± 2.06	7.66 ± 2.26	6.76 ± 2.20	*P* (1,2) =0.0160* *P* (1,3) =0.7082 *P* (2,3) =0.0464*
Sperm motility (%)	5.05 ± 1.41	4.85 ± 1.29	4.90 ± 1.51	*P* (1,2) =0.4611 *P* (1,3) =0.6088 *P* (2,3) =0.8591
Abnormal forms (%)	97. 94 ± 1.54	98.47 ± 1.83	98.26 ± 1.41	*P* (1,2) =0.1204 *P* (1,3) =0.2812 *P* (2,3) =0.5219

Result values represent by Mean ± SD. (*) $p < 0.05$ represents significant differences between groups.

The results of the present study showed that there was no significant difference in the percentages of sperm count, motility, and abnormality between the groups; the sperm counts in groups (1), (2), and (3) were 6.60±2.06, 7.66±2.26, and 6.76±2.20, respectively; these differences were statistically significant (P < 0.05). Groups (1), (2), and (3) had sperm motility incidences of 5.05±1.41, 4.85±1.29, and 4.90±1.51 respectively; these differences were not statistically significant (P < 0.05). The sperm abnormality differences in group (1) were 97.94±1.54. It was 98.47±1.83 in group (2) and 98.26±1.41 in group (3). P > 0.05 indicated that these differences were not statistically significant. Furthermore, as indicated in Table (3), the results demonstrated a comparison of the morphological categories of sperm abnormalities among the specimens examined in groups. Sperm head, midpiece, and tail defects were more common in group (1) than in groups (2) and (3), with incidences of 92.41 ± 3.80, 60.21 ± 4.81, and 17.71 ± 7.41, respectively, and 89.62 ± 4.51 and 89.82 ± 6.53, 58.81 ± 4.29 and 59.91 ± 5.14, and 15.92 ± 8.31 and 16.80 ± 8.22, respectively. P > 0.05 indicated that these differences were not statistically significant.

Table 3: Comparison between Sperms morphological analysis among studied male groups (n= 150)

Defects Categories	Group 1 (50)	Group 2 (50)	Group 3 (50)	Significance
Head defects	92.41 ± 3.80	89.62 ± 4.51	89.82 ± 6.53	*P* (1,2) =0.0012** *P* (1,3) =0.0172* *P* (2,3) =0.8589
Midpiece defects	60.21 ± 4.81	58.81 ± 4.29	59.91 ± 5.14	*P* (1,2) =0.1278 *P* (1,3) =0.7638 *P* (2,3) =0.2481
Tail defects	17.71 ± 7.41	15.92 ± 8.31	16.80 ± 8.22	*P* (1,2) =0.2584 *P* (1,3) =0.5623 *P* (2,3) =0.5957

Result values represent by Mean ± SD. (*) $p < 0.05$ represents significant differences between groups. (**) $p < 0.05$ represents highly significant differences between groups.

3.3.2. Oocyte Collection.

All groups had comparable numbers of oocytes that were collected, mature, and fertilized. To give background information about the female partners, these data are presented descriptively. Because the study groups were established based on the activation of sperm.

Table 4: Comparison between Oocyte factors in female partner among studied groups (n=150)

Oocyte factor	Group 1 (50)	Group 2 (50)	Group 3 (50)
Collected Oocyte	11.93 ± 2.23	11.89 ± 3.30	12.00 ± 2.04
Mature oocytes	9.03 ± 1.93	8.89 ± 2.10	8.90 ± 2.01

Data presented as mean ± standard deviation.

3.4. *Intracytoplamic sperm injection (ICSI)*

3.4.1. Evaluation of fertilization and cleavage

Comparing the cleavage rate and fertilization rates of the groups under study, represented in Table (5). The incidence of fertilization rates are highly significant ($P≤0.01$) in group (3), 78.40±10.06 then 73.00±9.13 in group (1) and in group (2), was 72.77±11.27. Also cleavage rate on day 2 and day 3 there is highly significant ($P≤0.01$) in group (3) by 76.30 ± 4.06 if compared with groups (1 and 2) which were 69.00± 4.02 and 70.17 ± 3.20. The cleavage rate incidence at day 5 (blastocyst formation rate) in group (1) was, 41.30 ± 6.33, In group (2), was 51.15 ± 5.31, highest in group (3), was 66.10± 5.56, these differences were statistically significant at ($P < 0.01$), as shown in Figure (3).

Table 5: Comparison between fertilization & Cleavage rates in female partner among studied groups (n=150)

Parameters	Group 1 (50)	Group 2 (50)	Group 3 (50)	Significance
Fertilization %	73.00 ± 9.13	72.77 ± 11.27	78.30 ± 10.06	*P* (1,2) =0.9109 *P* (1,3) =0.0069** *P* (2,3) =0.0111*
Cleavage on (D3)	69.00 ± 4.02	70.17 ± 3.20	76.30 ± 4.06	*P* (1,2) =0.1106 *P* (1,3) <0.0001** *P* (2,3) <0.0001**
Cleavage on (D5)	41.30 ± 6.33	51.15 ± 5.31	66.10 ± 5.56	*P* (1,2) <0.0001** *P* (1,3) <0.0001** *P* (2,3) <0.0001**

Result values represent by Mean ± SD. (*) $p < 0.05$ represents significant differences between groups. (**) $p < 0.05$ represents highly significant differences between groups.

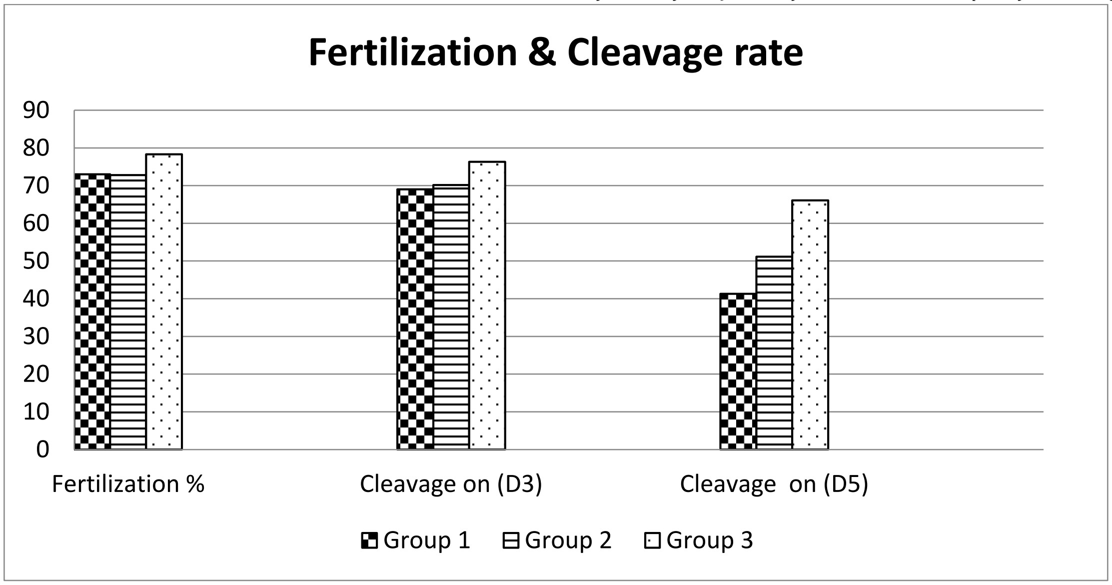

Fig. 3. Comparison between fertilization & Cleavage rates in female partner among studied groups.

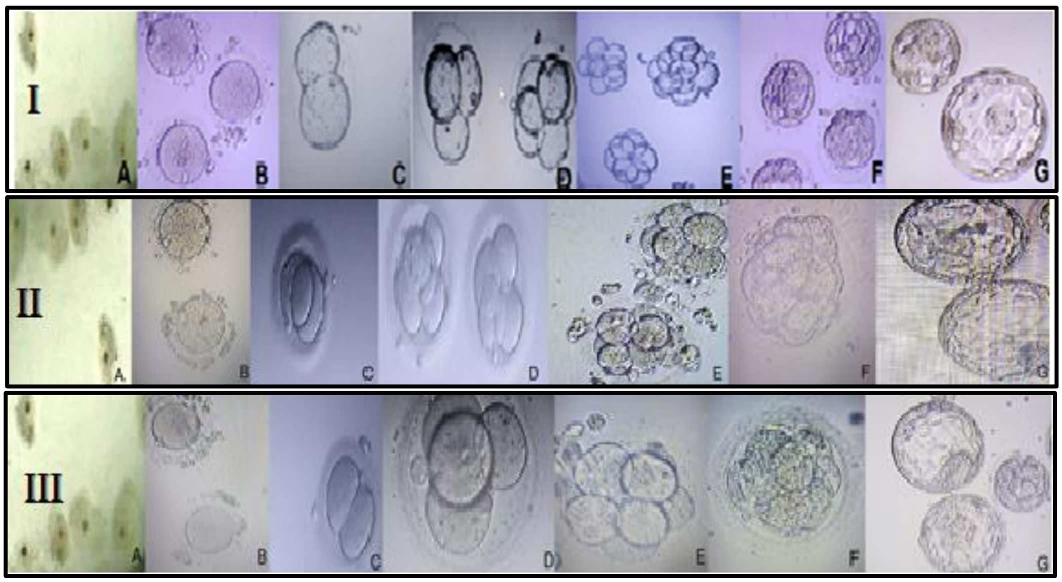

Fig. 4. Group (1): Pentatoxyphlline, II. Group (2): SpermMobil and III. Group (3): Biotin. A: Oocyte with corona cells, B: Mature oocytes, C: Embryo 2 cell stage, D: Embryo 4 cell stage, E: Embryo (8-10) stage, F: Embryo (morula) stage, and G: Embryo (blastocyst – expanded blastocyst).

3.4.2. Chemical Pregnancy

Incidence the status of chemical pregnancy among groups, represented in Figure (5). In group (1) pregnancy rates were 42.40±4.54, 46.50±3.61, and 49.10±3.47 in group (1,2 and 3) respectively. These results generally reveal that significant differences were found between (1, 2), (1, 3) at (*P* <0.01*) and non-significance between + ve chemical pregnancy between groups (2, 3) at *P*>0.05.

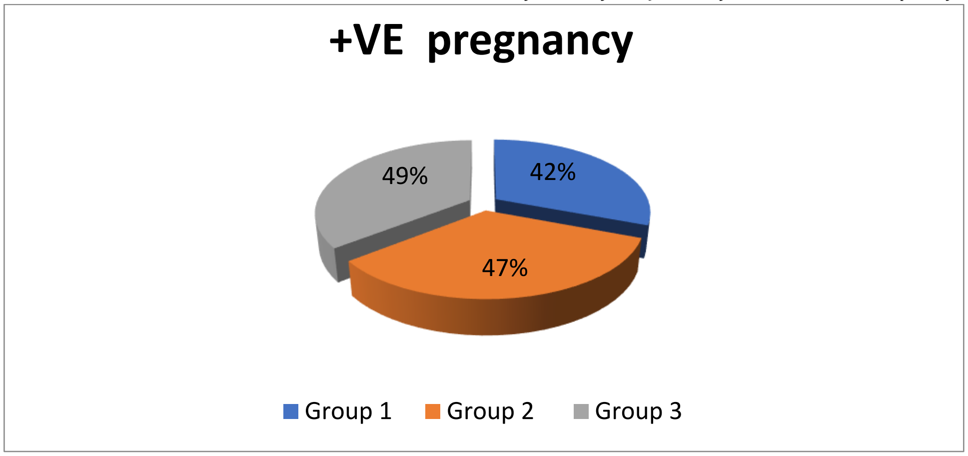

Fig. 5. Positive chemical pregnancy in female partner among studied.

4. Discussion

Biotin, known as vitamin B7 or vitamin H, has a critical importance in development and growth. Biotin deficiency is seldom recorded in humans; it can lead to teratogenesis in lower animals [24]. Additional research is required to fully understand how biotin affects sperm motility, including optimal dosages and application methods. Integrating biotin into standard preparations for ART procedures may significantly enhance reproductive health, particularly for couples facing infertility. Our study indicates that nuclear damage percentage in the biotin group compared to control groups is significantly decreased. Notably, a biotin concentration of 30 µM to 100 µM of semen (30:100 ratios) was found to be ideal, showing only 2.3% DNA damage, the lowest among the ratios assessed. This suggests a beneficial effect of biotin on sperm DNA integrity [25]. Sawamura et al. [26] reported that adding 3 µM biotin improved post-thaw sperm qualities and reduced lipid peroxidation, thereby protecting sperm structures and functions, including DNA and mitochondrial integrity. Conversely, excessive biotin can inhibit spermatogenesis in younger rats. Lipid metabolism is impacted by decreased activity of biotin-dependent enzymes, which may result in inherited lipid metabolism abnormalities [27]. The critical role of biotin in maintaining chromatin organization and preventing DNA damage is increasingly recognized. Zempleni and Hassan [28] have highlighted that teratogenic effects from biotin deficiency stem from chromosomal instability due to reduced biotinylated histones, with studies showing that multivitamin supplements containing biotin decrease the incidence of birth abnormalities [29]. These results reported that treatment with biotin are significantly increase the total and progressive motility of asthenozoospermic specimens. Velocity parameters such as curvilinear velocity (VCL), linear velocity (VSL), and average path velocity (VAP) also significantly improved. Chen et al. [30] corroborated these findings, showing that biotin supplementation in sperm wash medium enhances both motility and lifespan of frozen-thawed human sperms. Similarly Satish et a. [31] noted that biotin maintained higher progressive motility and in vitro survival rates in frozen-thawed semen.

Basic semen parameters assessed included sperm count, total mobility, and sperm abnormalities. No significant differences were found between groups treated with pentoxifylline, SpermMobil, and biotin. Our findings align with those of Rezaie et al. [32] who reported that both biotin and pentoxifylline enhance sperm motility in normozoospermic and asthenozoospermic samples, with the biotin group demonstrating superior in vitro survival. Enhanced motility enables for the extraction of more motile spermatozoa for therapeutic insemination, potentially leading to less invasive procedures such as transitioning from ICSI to IVF and IVF to intra-uterine insemination (IUI). Improved motility can help identify viable spermatozoa for ICSI in cases of absolute asthenozoospermia or frozen-thawed testicular tissue [33].Our results suggest that adding biotin to sperm media improves fertilization and cleavage rates, consistent with Kalthur et al.[34] who studied biotin's effects on fertilization ability and embryo development. Ranjan [25] supported that biotin in sperm wash medium enhances fertilization rates and blastocyst formation. Biotin's presence in embryo culture media also has a positive impact on preimplantation embryo growth. Previous research on biotin deficiency's teratogenic consequences suggests its nutritional importance during early embryo development. Concerns regarding pentoxifylline (PTX) include negative effects on oocyte and embryonic development [35]. Yet, our study highlighted better blastocyst and hatching rates with PTX compared to control, possibly due to the lower concentration used (1 mM). Variability in reports regarding PTX's toxicity may stem from differences in concentration; studies have used doses ranging from 1 to 5 mM [34]. Research encourages optimal PTX concentrations that improve motility of spermatozoa without harmful effects on embryonic development. Notably, higher concentrations of PTX have been linked to dramatically reduced blastocyst rates, while biotin, even at greater concentrations (up to 100 nM), and do not adversely affect embryonic development. Therefore, biotin's trace presence in sperm suspension can further benefit embryo development, supporting its nutritional role during early embryonic stages amidst findings of teratogenic effects caused by deficiency [36].

5. CONCLUSIONS

Biotin plays a significant role in enhancing sperm health, particularly in assisted reproductive technologies (ART). It improves sperm motility and DNA integrity while reducing oxidative stress. The optimal dosing of 30 µM of biotin with 100 µM of semen highlights the need for careful dose selection. Its association with increased fertilization rates suggests that biotin may be a safer alternative to pharmacological agents in sperm preparation. More research is needed to clarify its mechanisms and develop clinical guidelines for fertility treatments.

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Author Contributions

All authors contributed to this work. F. Abdulhadyl prepared the samples and completed the experimental measurements. Both E. Hassen and S. Mohamed shared writing and followed the performance of the experiments. M. Abdelrahman helped the first author complete the sample preparation. A. Kandeel with W. Tawfik completed the paper writing, analyzing the data, and validation. S. Mohamed and W. Tawfik followed the revision and submission of the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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