HUE UNIVERSITY
UNIVERSITY OF MEDICINE AND PHARMACY

NGUYEN THI HIEP TUYET

STUDY ON THE EFFECT OF SPERM DNA FRAGMENTATION AND SPERM SELECTION TECHNIQUE ON THE RESULTS OF IN VITRO FERTILIZATION

Major: Biomedical Science
Code: 9720101

SUMMARY OF THE THESIS

HUE – 2023

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INTRODUCTION

Tests that measure the degree of sperm DNA fragmentation are important in the diagnosis and finding of the cause of infertility. Some studies have shown that sperm DNA fragmentation is related to embryo quality, and fetal development, however, no association has been reported. In Vietnam, there have been some studies on sperm DNA fragmentation and outcome of in vitro fertilization. However, with a small sample size and no in-detail assessment of the quality of embryos at each stage of development.

Physiological sperm selection is a technique to obtain mature spermatozoa based on the characteristic that mature spermatozoa have specific receptors for hyaluronic acid, while immature spermatozoa do not have receptors. Sperm selection techniques can optimize sperm intracytoplasmic sperm injection results by selecting mature, DNA fragment-free sperm that improves embryo outcomes for in vitro fertilization. However, there are not many studies analyzing the effectiveness of this technique. In Vietnam, there is currently only 1 study reporting on the use of the hyaluronic acid-containing medium to select sperm.

At the Center for Reproductive Endocrinology and Infertility, Hue University of Medicine and Pharmacy Hospital, during the examination and treatment of infertile couples, there were many semen samples with high DNA fragmentation. We study with the desire to provide more specific scientific evidence on how does sperm DNA fragmentation effect the ability to fertilize, embryo quality, and embryo transfer results? In addition, we apply a new technique called the physiological sperm selection technique to inject sperm into the oocyte cytoplasm. Will the results of the study demonstrate whether this technique has a practical effect on embryogenesis? With the above research questions, we studied: **" Study on the effect of sperm DNA fragmentation and sperm selection techniques on the results of in vitro fertilization "**

With objectives:

1. Determine the relationship between sperm DNA fragmentation with semen parameters, embryo quality, and in vitro fertilization results.

2. Evaluation of the impact of physiological sperm selection techniques on the results of in vitro fertilization embryogenesis.

NEW CONTRIBUTIONS OF THE THESIS

The study determined the relationship between sperm DNA fragmentation and some semen parameters in male infertile couples in Vietnam. The results showed a number of associations and correlations between the degree of sperm DNA fragmentation and fertilization rate, the characteristics of day 2 division, and blastocyst.

Evaluation of the impact of sperm selection techniques based on comparison of fertilization and embryo development on day 2 and blastocyst, when simultaneously two techniques, including the physiological sperm selection to inject sperm into the oocyte cytoplasm and the routine intracytoplasmic with sibling oocytes in each cycle. This is a study using a dedicated PICSI disc to select physiological sperm. In PICSI, the rate of grade 1 blastocyst formation was lower than that of ICSI, but the rate of grade 2 and 3 blastocyst formation in PICSI was statistically significantly higher than that of ICSI.

STRUCTURE OF THESIS

Thesis includes 121 pages

- Introduction	2 pages
- Chapter I: Literature review	42 pages
- Chapter II: Methodology	21 pages
- Chapter II: Results	28 pages
- Chapter IV: Discussion	25 pages
- Conclusion	2 pages
- Recommendation	1 page

The thesis includes 38 tables, 18 pictures, 4 figures and 173 references (in which, 16 Vietnamese references, 157 English references), 5 journal articles related to the thesis.

Chapter 1
LITERATURE REVIEW

1.1. ASSESSMENT OF MEN'S FERTILITY

Semen analysis is a test to assess the quality of sperm, through indicators such as quantity, motility, and normal morphology ... based on the results of a semen analysis, one can make a general assessment of male fertility. Assisted reproductive units and semen analysis

laboratories are based on the guidelines of the World Health Organization (WHO).

1.1.1. Semen analysis

This is a method of sperm quality survey commonly applied in practice to evaluate male fertility, including General assessment including lysis, viscosity, volume, and pH; Microscopic examination includes motility, sperm survival rate, concentration, and sperm morphology.

1.1.2. Sperm DNA fragmentation test

Th e integrity of sperm DNA plays an important role in the processing of paternal genetic information into the ovum during fertilization, DNA fragmentation is the breakage of single- and double-stranded DNA.

Sperm DNA structure

Human sperm is a highly organized unit, the sperm chromosome is composed of three structural regions: (1) the majority of the sperm DNA is coiled into DNAse-insensitive toroids each toroid contains about 50kb of DNA; (b) A smaller amount of DNA is associated with histones present in the spermatozoa (3) the remaining DNA attached to the nuclear matrix at Matrix Attachment Regions.

Sperm DNA fragmentation and the causes of sperm DNA fragmentation

DNA fragmentation is the breakage of single-stranded and double-stranded DNA. The causes of sperm DNA fragmentation are multifactorial and can be divided into intrinsic and extrinsic factors. The primary cause emerges from the molecular level, some pathophysiological causes arise during spermatogenesis leading to DNA fragmentation. There are 3 main groups of causes leading to sperm DNA fragmentation, which can occur during spermatogenesis or in sperm motility in the genital and female tracts: (1) DNA fragmentation in the spermatogenesis; (2) Incomplete cell apoptosis of germ cells; (3) Consequences of exposure to reactive oxygen species (ROS).

Common methods for assessing sperm DNA fragmentation

Comet assay

The sperm chromatin structure assay

TUNEL assay

Dispersion of sperm chromatin assay

1.2. DEVELOPMENT CHARACTERISTICS OF EMBRYOS IN VITRO FERTILIZATION

1.2.1. Fertilization characteristics

Fertilization usually goes through the following stages: (1) sperm penetrate through the cumulus cell layer, (2) sperm perform the acrosome, (3) spermatozoa cross the transparent membrane, (4) the sperm comes into contact with the oocyte plasma membrane, which initiates the activation of the oocyte to carry out the fusion membrane, (5) the lysosome reaction occurs leading to the transparent membrane reaction, and (6) the formation of two pronuclei. An oocyte is considered to be fertilized normally when two pronuclei are present. Usually, both progenitors appear at the same time between 16 and 20 hours after fertilization with a mature oocyte.

1.2.2. Characteristics of embryos in the division stage (days 2 - 3)

After the 2-cell stage, the zygote has more mitosis that increases the number of cells called division with cells that get smaller with each division, called blastomeres, and the total block size of cells did not change. In the evaluation of division stage embryos, the best observational timelines by Alpha consensus are as follows: Day 1: (26 ± 1) hours after ICSI, (28 ± 1) hours after IVF: 2 cells plankton; Day 2: (44 ± 1) hours, 4 cells; Day 3: (68 ± 1) hours, 8 cells. When evaluating embryo selection, several factors are often combined: embryo development rate, embryo morphology such as the number of fragments, thickness of the transparent membrane, blastocyst development, and the number of nuclei cell.

1.2.3. Morula embryo (embryo day 4)

In humans, the morula begins to form when the embryo is at the 8 blastocyst stage and begins the compaction process. The process of compaction is a process of forming tight bonds between blastomeres, the part of blastomeres in contact with each other increases and flattens out to form a mass with no clearly visible boundaries between blastomeres. As compaction increases, the boundaries between blastomeres become indistinguishable as the blastomeres flatten out and join together. The morula at this stage completely looks like a multinucleated cell.

1.2.4. Blastocyst (embryos day 5-6)

The blastocyst usually forms about 100 hours after fertilization. After 5-6 days of culture, 26-65% of embryos will develop to this

stage. During blastocyst formation, two types of blastomeres are formed: germ cells and trophoblasts. The trophoblast is the first type of embryo to be differentiated during embryogenesis. These two types of blastomeres become increasingly different as they move to new locations during blastocystogenesis. The trophoblasts are oval and polar, while the embryo blasts have remained round and morphologically unchanged. The trophoblasts are connected by small surface contacts, while the embryo blasts are in close contact to form a mass. The blast blasts migrate toward one pole of the embryo called the inner cell mass, which is tightly bound together and has pluripotent properties.

1.3. PHYSIOLOGICAL SPERM SELECTION TECHNIQUE

Hyaluronic acid is present in the cumulus cell complex around the oocyte. Only mature spermatozoa, which have completed the rearrangement of functional structures, have receptors for hyaluronic acid in the sperm cell membrane. Therefore, when the sperm is attached to the cumulus cell complex around the oocyte, it can go through the next steps of the natural fertilization process. At present, several advanced methods of sperm selection have been developed to follow the mechanisms of natural selection. Among them, sperm selection based on the maturation of the cell membrane at the sperm head is being widely used, sperm selected from this principle will be used to perform ICSI (called Physiological ICSI - PICSI). This method, which selects sperm with high DNA integrity and maturity, has been shown to be effective in groups with high DNA damage.

Authors Huszar et al suggest that the determination of the hyaluronic acid binding index based on the sperm hyaluronic acid binding assay (HBA) can be used to predict the success of the assisted reproductive technology.

Sperm selection was performed based on the ability of sperm to bind to hyaluronic acid at the hyaluronic acid micropoints on the surface of the PICSI disc, when the filtered sperm was washed into the hyaluronic acid site and incubated for 8-10 minutes, attachment will occur at the head of the sperm when observed under the microscope, the sperm adheres to the bottom of the plate and the sperm motile tail at the site of attachment. Selection of spermatozoa in situ to perform sperm injection into the cytoplasm of the oocyte, so-called PICSI technique - physiological sperm selection technique.

1.4. RELATED STUDIES

1.4.1. Study on the relationship between sperm DNA fragmentation and semen parameters

In the world

Sivanarayana et al (2014) evaluated by chromatin dispersion technique, reported significantly higher mean sperm count in the DFI group < 30% compared with the DFI group ≥ 30%. Fast-progressive and slow-progressing motility in the DFI <30% group was significantly higher than in the DFI ≥30% group, respectively (21.40 ± 11.53 vs 13.58 ± 11.31) (31.23 ± 14.97 vs 22.37 ± 12.70) ($p < 0.001$).

Author Borges et al (2019) used the chromatin dispersion technique, and the percentage of men with DFI ≥30% is 8.84%. The DFI group < 30% had a higher percentage of advanced motile sperm compared with the DFI ≥30% group (54.90 ± 14.27% vs 46.50 ± 16.77%, $p < 0.001$).

In Vietnam

In Vietnam, the research team of author Le Minh Tam (2019), evaluated 318 men from infertile couples at the Center for Endocrinology and Reproductive Medicine, Hue University of Medicine and Pharmacy hospital. The results recorded that the level of sperm fragmentation was positively correlated with the percentage of sperm head abnormalities ($r = 0.202$, $p = 0.0003$) and negatively correlated with progressive motility ($r = - 0.168$, $p = 0. 0027$).

Research by author Duong Thi Nhan (2020) evaluates sperm DNA fragmentation by surveying the structure of sperm chromatin on 151 sperm samples. The DFI value was 21.58 ± 15.53%, there was an inverse correlation between DFI with semen pH ($r = - 0.02$ and $p = 0.012$), and sperm concentration ($r = - 0.02$). - 0.26, $p = 0.02$), sperm survival rate ($r = - 0.32$ and $p < 0.001$).

1.4.2. Study on the relationship between sperm DNA fragmentation and in vitro fertilization results

In the world

In 2017, a study by Alvarez Sedo et al. Evaluated sperm DNA fragmentation by the TUNEL technique on ICSI results, and the results had a negative correlation ($r = - 0.5$) between the degree of DNA fragmentation and the ratio. Blastocyst formation rate, however, fertilization rate was not affected. Sperm DNA fragmentation is negatively correlated with blastocyst and pregnancy rates even in

good-quality sites. The results of Zheng et al in 2018 showed that sperm DNA fragmentation did not significantly affect fertilization results, but significantly reduced the rate of blastocyst development and pregnancy rate.

In Vietnam

The author Nguyen Minh Tai Loc et al (2016), fertilization rate in the group of patients with DFI index > 15% were statistically significantly lower than in the group of patients with DFI ≤ 15% (91% vs 84%; p=0.03). No statistically significant correlation was found between the DFI index and embryo quality. Author Nguyen Thi Quynh Tien et al in 2018, there was no correlation between DNA fragmentation index and ICSI results, including fertilization rate, embryogenesis rate on day 3, and rate of useful embryos on day 3 (p> 0.05). Clinical pregnancy in the three DFI groups had no difference.

1.4.3. Study on the effect of sperm selection technique on the outcome of in vitro fertilization

In the world

In the study by Parmegiani et al., 2010 reported a higher overall embryogenesis rate in the PICSI group (using hyaluronic acid-based SpermSlow medium) (95.0 ± 0.8) compared with the ICSI group (84.0 ± 1.1, p < 0.001). The percentage of best-quality embryos in the PICSI group was higher than in the ICSI group (35.8% vs 24.1%, p < 0.046).

Author Choe et al (2012) evaluated on 219 oocytes from 18 women who performed PICSI (n = 107)/ICSI (n = 112) halves of the oocyte. Results of fertilization rate and day 2 embryos recorded in PICSI were lower than in the ICSI group, but the difference was not statistically significant. The percentage of embryos on day 3 was significantly lower in the PICSI group (56.0% vs 69.6%, p = 0.038). The blastocyst formation rate and the number of embryos transferred were similar in both groups.

In Viet Nam

Research by Le Thuy Hong Kha, 350 cycles using hyaluronic acid medium - SpermSlow, compared with 350 cycles of conventional ICSI. There was no statistically significant difference between the two groups in terms of fertilization rate, number of good embryos. The clinical pregnancy rate and implantation rate of the PICSI group were not different from that of the ICSI group. The PICSI group had a higher rate of ongoing pregnancy than the ICSI group, this difference was statistically significant.

Chapter 2
METHODOLOGY

2.1. STUDY SUBJECTS

Couples treated for infertility by in vitro fertilization (convenient ICSI or parallel PICSI and convenient ICSI) and at the Center for Reproductive Endocrinology and Infertility, Hue University Hospital of Medicine and Pharmacy from 1/2019 to 3/2022.

2.1.1. Inclusion criteria

Objective 1

- Wife: full of clinical and preclinical information. Results of the number of oocytes aspirated ≥ 2 oocytes, with mature oocytes MII to perform ICSI.

- Husband: full clinical and laboratory information, semen analysis, sperm DNA fragmentation test.

- In vitro fertilization technique: by ICSI technique, with results according to fertilization, embryo development day 2, blastocyst, and blastocyst transfer result (if any).

Objective 2

- Wife: full of clinical and preclinical information. As a result, the number of eggs aspirated was ≥ 10 oocytes, there were enough mature MII oocytes to split the number of oocytes to perform PICSI and ICSI techniques.

- Husband: full clinical and laboratory information, semen analysis, sperm DNA fragmentation test.

- In vitro fertilization technique: by PICSI and ICSI techniques, with results according to fertilization, embryo development day 2, blastocyst.

2.1.2. Exclusion criteria

Objective 1

- The husband has a varicocele, urogenital infection, retrograde ejaculation, or a history of varicocele surgery, testicular disease, an inguinal hernia, and inability to ejaculate. Sperm samples that have been cryopreserved or obtained from testicular surgery; patients with very low sperm count (less than 1 million/mL) or azoospermia.

- In vitro fertilization cycles with donor sperm (donor sperm in cryopreservation is not included in the research method); or ask for an oocyte donation.

- The cases of embryo culture did not evaluate the embryo stage on day 2 and day 5.

Objective 2

- Exclusion criteria as goal 1, in addition, patients with very low sperm count (less than 5 million/mL)
- In vitro fertilization cycles with donor sperm (donor sperm in cryopreservation is not included in the research method); or ask for an oocyte donation.
- In case the number of oocytes collected is less than 10.
- The cases did not evaluate the embryo stage on day 2 and blastocyst

2.2. METHODS AND SAMPLE

2.2.1. Study design

Objective 1:

- Study design: Cross-sectional descriptive study
- Study sample size: according to the formula for calculating sample size for cross-sectional descriptive research

The study collected 242 couples who met the study criteria.

Objective 2:

- Study design: Similarity comparison intervention study
- Study sample size: Convenient sample size selection method. The study results obtained 74 couples who met the research criteria.

2.2.2. Research variables and indicators

Variables, indicators of general characteristics of research subjects

Variables, indexes of semen parameters, sperm DNA fragmentation

Sperm parameters (according to WHO 2010 guidelines)

Sperm DNA fragmentation

- Sperm DNA fragmentation index: DFI
  + DFI < 15%: Low level
  + 15% DFI < 30%: Moderate
  + DFI 30%: High level

Sperm-binding hyaluronic acid

- Sperm index that binds hyaluronic acid
- Grouping level of HBA 60%, HBA > 60%

Variables, indicators of results of in vitro fertilization

Objective 1

- Number of mature oocytes
- Fertilization rate (%)
- The result of cleavage embryo formation on day 2
  + Rate of embryo cleavage on day 2 (%)
  + Rate of embryos cleavage grade 1 (%)
  + Rate of useful embryos on day 2/zygote (%)
  + Rate of useful embryos on day 2/MII oocytes (%)
  + The rate of embryos < 4 cells, 4 cells, > 4 cells (%)
  + Rate of cytoplasmic fragmentation: 0 -< 10% , 10% - ≤25%,>25%
- Results of blastocyst formation:
  + The rate of blastocyst/embryo cleavage day 2 (%)
  + The rate of blastocyst grade 1 / embryo day 2 (%)
  + The rate of blastocyst/zygote (%)
  + The rate of blastocyst/oocyte MII (%)
- Embryo transfer results
  + Number of embryos transferred
  +Thickness of the uterine lining on the day of embryo transfer (mm)
  + βhCG, gestational sac, ongoing pregnancy 12 weeks: yes/no

Objective 2

- Number of MII oocytes, number of oocytes fertilized at PICSI – ICSI
- Total number of day 2 cleavage embryos formed at PICSI – ICSI
- Number of embryos of grade 1, grade 2, in PICSI – ICSI
- Number of embryos < 4 cells, 4 cells, > 4 cells in PICSI – ICSI
- The number of embryos with cytoplasmic fragmentation: 0 -< 10% , 10% - ≤25%, >25% in PICSI – ICSI
- Number of blastocysts, number of grade 1 blastocysts, number of grade 2 and 3 blastocysts in PICSI – ICSI

2.2.3. Procedure to proceed

Data were collected based on questionnaires. All infertile couples are examined and treated for in vitro fertilization according to the following procedure:

Clinical and subclinical examination

Semen analysis, sperm DNA fragmentation

Semen analysis

According to WHO guidelines 2010

Sperm DNA fragmentation test

Chemicals and tools: Halosperm. kit

Semen samples were collected and processed according to the manufacturer's instructions for Halosperm Kit, according to the manufacturer's procedures.

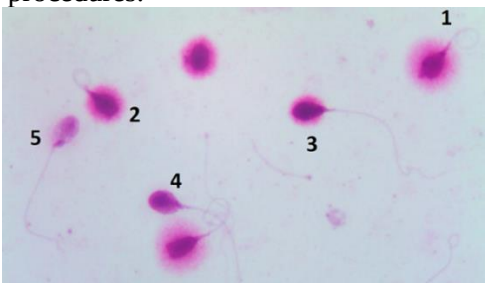

Figure 2.1. Sperm imaging in chromatin dispersion test

1: Sperm with big halo; 2: Sperm with medium halo; 3: Sperm with small halo; 4: Sperm without halo; 5: Sperm degeneration

- Formular to calculate DFI: DFI (%) = [(small halo + without halo + degenerate)/(500 sperms)] x 100

In vitro fertilization techniques

- Ovarian stimulation and oocyte retrieval
- Sperm washing technique in preparation for in vitro fertilization
- In vitro fertilization: ICSI
- PICSI technique - Sperm selection
- Evaluation of fertilization and embryo development: According to Alpha consensus (2011)
- Embryo transfer and follow-up after embryo transfer

2.2.4. Analyze and process data

Data was entered and analyzed on SPSS 20.0 software, ensuring accuracy.

Evaluate the correlation between DFI and sperm index, between DFI and embryo results by Pearson test. Compare the mean values between 2 groups classified by Independent Sample Test. Compare the mean values among 3 groups by the Anova test. Compare the fertilization rate, embryo formation rate on day 2, blastocyst, and embryo transfer results between two groups PICSI and ICSI by Chi-Square test. The difference was statistically significant with $p \leq 0.05$.

2.3. ETHICS OF STUDY

The research is approved by the Professional Council. The study was approved by the Ethics Committee in Biomedical Research Hue University of Medicine and Pharmacy. File number: H2020/030.

Chapter 3
RESULTS

3.1.THE RELATIONSHIP BETWEEN SPERM DNA FRAGMENTATION WITH SEMEN PARAMETERS, EMBRYO QUALITY, AND IN VITRO FERTILIZATION RESULTS.

3.1.1. General characteristics

3.1.1.1. Wife characteristics

The average age of the wife in reproductive age, the elderly wife (≥ 35 years old) accounted for 26.4%. The average number of mature oocytes obtained in each ICSI cycle was 12.02 ± 7.29.

3.1.1.2. Husband characteristics

The average age of the husband is 35.57 ± 5.24. The mean level of sperm DNA fragmentation in infertile couples was 23.65±13.80 %. The levels of sperm DNA fragmentation with low (DFI<15%), medium (15%≤DFI<30), and high (DFI ≥30%) were: 27.7%, 47.9%, and 24.4%, respectively.

3.1.2. The relationship between sperm DNA fragmentation and semen parameters

Table 3.8. The relationship between semen parameters in sperm DNA fragmentation groups

Sperm DNA fragmentation / Sperm parameters	DFI<15% n= 67	15%≤DFI<30% n= 116	DFI ≥30% n=59	p
pH	7,43±0,51	7,43±0,48	7,19±0,41	0,04
Volume (mL)	2,46±1,08	2,64±1,40	2,18±1,24	0,09
Progressive (%)	30,40±8,86	26,29±10,98	27,66±12,86	0,05
Concentration (10^6/mL)	34,45±15,52	38,16±19,50	30,62±16,95	0,03
Survival rate (%)	84,22±9,63	81,41±9,02	80,44±8,41	0,04
Normal morphology (%)	3,64±2,04	3,48±1,90	3,07±2,22	0,26
Head abnormality	90,19±5,54	91,32±5,56	89,88±6,54	0,23
Neck and tail abnormalities (%)	50,24±8,77	51,22±9,10	55,76±12,84	0,005
DFI	11,19±2,59	20,81±3,75	43,38±12,82	

There were differences in pH, motility, concentration, survival, sperm neck and tail abnormalities between the 3 groups of DFI levels.

Table 3.12. Correlation between sperm DNA fragmentation and sperm parameters

Sperm parameters	DFI	
	r	p
pH	-0,21	0,001
Volume (mL)	-0,12	0,07
Progressive (%)	-0,11	0,08
Concentration (10^6/mL)	-0,12	0,06
Survival rate (%)	-0,14	0,03
Normal morphology (%)	-0,13	0,04
Head abnormality (%)	-0,03	0,62
Neck and tail abnormalities (%)	0,23	0,00

There was a weak negative correlation between the pH of semen and the percentage of survival sperm; normal morphology with DFI.

3.1.3. The relationship between sperm DNA fragmentation with embryo quality, and in vitro fertilization results.

3.1.3.1. Relationship between sperm DNA fragmentation and fertilization results, embryo cleavage results on day 2

Table 3.13. Relationship between sperm DNA fragmentation and fertilization results, embryo cleavage results on day 2

Sperm DNA fragmentation / Embryo	Mean	DFI<15% n= 67	15% ≤ DFI <30% n= 116	DFI ≥30% n=59	p
Fertilization rate (%)	72,59± 17,64	75,14 ±15,90	73,12± 18,37	68,66± 17,69	0,11
The result of cleavage embryo formation on day 2 (%)					
Rate of embryos on day 2/zygote	95,98± 9,32	97,32± 5,56	94,73± 11,41	96,92± 7,87	0,13
Rate of useful embryos on day 2/MII	65,35± 25,10	67,94 ±23,82	64,77± 29,51	63,57± 29,51	0,59
Rate of useful embryos on day 2/zygote	81,05± 21,40	82,60± 19,05	80,71± 21,60	79,94± 23,68	0,19
Useful embryos on day 2/MII	59,18± 22,04	62,38± 20,02	59,36± 22,91	55,19± 22,21	0,77

Sperm DNA fragmentation / Embryo	Mean	DFI<15% n= 67	15% ≤ DFI <30% n= 116	DFI ≥30% n=59	p
Characteristics of cleavage embryos day 2 (%)					
Embryos < 4 cells	22,32± 24,91	24,68± 22,77	21,81± 22,47	20,66± 24,10	0,58
Embryos 4 cells	59,16± 26,29	54,8851± 26,42	61,65± 25,88	59,14± 26,79	0,25
Embryos > 4 cells	18,75± 19,89	20,73± 19,53	17,05± 19,33	19,85± 21,42	0,43
Cytoplasmic fragmentation 0 - < 10%	82,61± 22,78	88,17± 18,67	82,92± 20,64	75,67± 28,80	0,008
10% ≤ Cytoplasmic fragmentation ≤ 25%	14,22± 18,19	11,18± 16,23	14,28± 16,55	17,56± 22,59	0,14
Cytoplasmic fragmentation >25%	3,21± 10,23	1,75 ±5,72	2,60± 7,88	6,11± 16,21	0,04

The DFI group ≥30% had the lowest fertilization rate, p = 0.11. The rate of useful embryos on day 2/MII oocytes in the DFI group ≥30%had the lowest value, p=0.77.

Table 3.14. Correlation between DFI and fertilization outcome and day 2 cleavage embryos

	DFI	
	r	**p**
Fertilization rate	-0,20	0,002
The result of embryo formation on cleavage day 2		
Rate of embryos on day 2/zygote	0,01	0,87
Rate of embryos grade 1 on day 2/zygote	-0,08	0,21
Rate of useful embryos on day 2/zygote	-0,07	0,30
Rate of useful embryos on day 2/MII	-0,16	0,01
Characteristics of cleavage embryos day 2		
The rate of embryos < 4 cells	-0,02	0,72
The rate of embryos 4 cells	0,05	0,44
The rate of embryos > 4 cells	-0,04	0,48
Cytoplasmic fragmentation 0 -< 10%	-0,17	0,008
10% ≤ Cytoplasmic fragmentation ≤ 25%	0,09	0,16
Cytoplasmic fragmentation >25%	0,18	0,006

There was a weak negative correlation between DFI and fertilization rate, rate of useful embryos on day 2/MII, cytoplasmic fragmentation < 10% and weakly positively correlated with cytoplasmic fragmentation rate > 25%

3.1.3.2. Relationship between sperm DNA fragmentation and blastocyst

Table 3.15. Relationship between sperm DNA fragmentation and blastocyst

DFI / Embryo results	Mean	DFI<15% n= 67	15% ≤ DFI <30% n= 116	DFI ≥30% n=59	p
Blastocyst/embryo cleavage	63,86± 25,61	66,89± 22,20	62,41± 25,80	63,27± 28,79	0,51
Blastocyst grade 1 / embryo day 2	35,23± 25,45	38,58± 23,22	33,65± 25,88	34,55± 27,03	0,44
Blastocyst/MII	44,27± 20,89	48,59± 19,00	43,35± 22,02	41,19± 20,17	0,11
Blastocyst/zygote	60,89± 24,56	65,20± 22,21	58,35± 24,03	60,98± 27,64	0,19

The DFI group ≥30% had the lowest rate of useful blastocyst formation /MII oocyte, (p=0.11).

Table 3.16. Correlation between DFI and blastocyst

Blastocyst	DFI	
	r	p
The rate of blastocyst/embryo cleavage day 2	-0,04	0,51
The rate of blastocyst grade 1 / embryo day 2	-0,41	0,53
The rate of blastocyst/MII	-0,15	0,02
The rate of blastocyst/zygote	-0,04	0,48

There was a weak negative correlation between DFI and the blastocyst/oocyte MII (r = -0.15, p=0.02).

3.1.4. Evaluation of the relationship between sperm DNA fragmentation and embryo transfer results

Out of 242 ICSI cycles, 204 transfer cycles. The rate of positive βhCG: 105/204, 51.5%; the number of cases with gestational sac: 95/204, 46.6%; The number of ongoing pregnancy cases: 80/204, 39.2%.

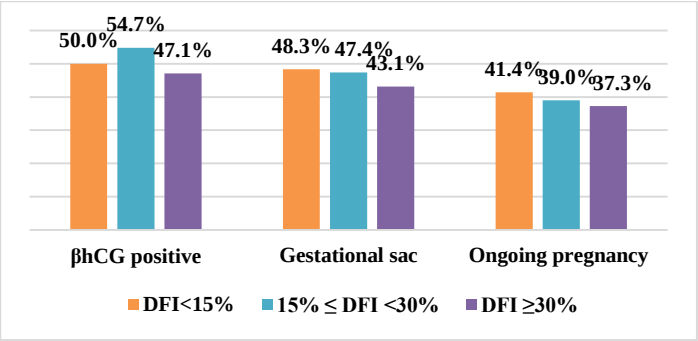

Chart 3.4. Results after embryo transfer in sperm DNA fragmentation groups

The rate of pregnancy in the DFI group ≥ 30% was the lowest among the 3 groups.

3.2. THE IMPACT OF PHYSIOLOGICAL SPERM SELECTION TECHNIQUES ON THE RESULTS OF IN VITRO FERTILIZATION EMBRYOGENESIS.

The results obtained were 74 infertile couples who met the selection criteria.

3.3.1. Sperm sample characteristics

After washing: sperm concentration reached 23.96 ± 8,88 x10^6/mL, progressive motility: 83.96±9.94%, and average HBA was 48.16±28.99%, in which the number of samples with low HBA: HBA ≤60% with 58.1%.

3.2.2. Results of in vitro fertilization of PICSI and ICSI techniques

3.2.2.1. Embryo culture results of PICSI and ICSI techniques

Table 3.25. Comparison of embryo culture results between the two techniques PICSI and ICSI

Two techniques / Embryo results	PICSI	ICSI	p
MII oocyte	585	596	
Fertilization (n;%)	434; 74,2%	447; 75,00%	0,75
The result of cleavage embryo formation on day 2	**/434**	**/447**	
Cleavage embryo (n;%)	413; 95,2%	432; 96,6%	0,27
Embryos of grade 1 (n;%)	263; 60,6%	291; 65,1%	0,17
Embryos of grade 2 (n;%)	86; 19,8%	79 ; 17,7%	0,41

Two techniques / Embryo results	PICSI	ICSI	p
Characteristics of cleavage embryos day 2	**/413**	**/432**	
Embryos < 4 cells	94; 22,8%	82; 19,0%	0,18
Embryos 4 cells	241; 58,4%	252; 58,3%	0,99
Embryos > 4 cells	78; 18,9%	97; 22,5%	0,20
Cytoplasmic fragmentation 0 - < 10%	324; 78,5%	345; 79,9%	0,61
10% ≤ Cytoplasmic fragmentation ≤ 25%	76; 18,40%	67; 15,5%	0,26
Cytoplasmic fragmentation >25%	13; 3,1%	20; 4,6%	0,27
Blastocyst	**/413**	**/432**	
Blastocyst	256; 62,0%	251; 58,1%	0,25
Blastocyst grade 1	114; 27,6%	143; 33,1%	0,08
Blastocyst grade 2 and 3	142; 34,4%	108; 25,0%	0,003

There was no difference in fertilization rate, embryogenesis rate of day 2 clevage, and embryo characteristics between PICSI and ICSI groups (p>0.05). In the PICSI group, the rate of grade 1 blastocyst formation was lower than that in the ICSI group, the difference tended to be statistically significant: 27.6% versus 33.10%, p=0.08. The rate of blastocyst formation grade 2 and 3 in the PICSI group was statistically significantly higher than in the ICSI group: 34.4% versus 25.0%, p = 0.003.

Chapter 4
DISCUSSION

4.1. THE RELATIONSHIP BETWEEN SPERM DNA FRAGMENTATION WITH SEMEN PARAMETERS, EMBRYO QUALITY, AND IN VITRO FERTILIZATION RESULTS.

4.1.1. The relationship between sperm DNA fragmentation and semen parameters

*4.1.1.1. Characteristics **of** sperm DNA fragmentation*

This study recorded that the average DFI value of infertile couples was 23.65±13.80%, this result is almost similar to 2 studies in

Vietnam in the North and the South on sperm samples of infertile couples: Duong Thi Nhan (2020), 21.58 ± 15.53% and Ho Manh Tuong (2021), 19.16 ± 13.68%. These two studies carried out the sperm chromatin structure assay, while we applied the chromatin dispersion assessment technique. The proportion of men with DFI ≥ 30% in this study was 24.4%; the proportion of men with DFI % ≥ 30% accounting for about 19 – 25% were also reported in studies in Vietnam.

4.1.1.2. ***The relationship between sperm DNA fragmentation and semen parameters***

pH, semen volume, concentration

In our study, the mean value of pH in the DFI group ≥ 30% (7.19±0.41) was statistically significantly lower than in the two groups with low and moderate DNA fragmentation. In addition, when analyzing the correlation between pH and the degree of sperm DNA fragmentation, the results showed a weak negative correlation with DFI (r = - 0.21, p = 0.001). Garcia-Segura S et al (2020) reported that pH was negatively correlated with viscosity-corrected oxidation of semen samples (p= - 0.347, p < 0.05), there was no correlation. Regarding the level of oxidation adjusted for sperm density and pH, the lower the pH, the higher the level of oxidation, leading to oxidative stress, which can cause damage to sperm DNA.

The difference in semen volume between groups in the degree of sperm DNA fragmentation, observed in samples with DFI ≥ 30% had less semen volume than samples with DFI < 30%, The difference was not statistically significant with p=0.09, and there was no correlation between DFI and semen volume. Most studies did not find a relationship between volume and DFI, but in Borges' study, there was a statistically significant lower semen volume in the DFI <30% group than in the DFI group. DFI ≥30% (2.94 ± 0.50 vs 3.79 ± 1.09 mL, p= 0.001.

The results of the study in the high DFI group had a statistically significant lower sperm density than the low and medium DFI groups, with p = 0.03. However, no correlation between the two indexes was recorded. this. Some other studies have recorded a strong correlation between sperm DNA fragmentation and density: Osaka A (2022) recorded a negative correlation between DFI and sperm density with r = −0.416, p < 0.001. In the study of Duong Thi Nhan, there was a negative correlation with r = - 0.26, p = 0.02. Specifically, the group <15,106/mL had a rather high mean DFI value of 26.66 ± 18.77%, of which the group ≥ 15,106/mL had a mean DFI 19.43 ± 13.46%, p = 0.008.

Sperm motility

The association of sperm DNA motility and fragmentation was also reported in many studies: mobility towards the DFI group < 30% was significantly higher than that in the DFI group ≥ 30% (54.90). ± 14.27 compared with 46.50 ± 16.77, p = 0.001) in Borges' study (2019). The results of the author Vinnakota C et al (2019) showed that the percentage of motile sperm in the groups with low, medium, and high levels of DNA fragmentation was 55.3 ± 15.8, respectively; 50.2 ± 15.2; 38.8 ± 16.1 (statistically significant difference). Research in Vietnam by author Ho Manh Tuong also found that DFI was negatively correlated with sperm motility (r = -0.477, p < 0.001). The group of patients with low DFI had a higher percentage of motile sperm than the other two groups (54.20 ± 13.61% vs 41.14 ± 15.82% and 43.21 ± 15.11%, p < 0.001). In our study, there was an association between DFI and forward mobility, the results showed that the progressive mobility in the DFI sample < 15% was faster than the two groups of average and High. It can be seen that the samples with good motility have less DNA fragmentation. These demonstrations suggest that the selection of fast-moving spermatozoa in ICSI is an approach to selecting sperm with high DNA integrity.

Sperm morphology

When analyzing the correlation, it was found that there is an inverse correlation between DFI and normal shape and there is a weak positive correlation with tail neck abnormality, respectively r=-0.13, p=0.04, and r = 0.23, p = 0.00. Recent studies also noted the correlation, reported by Osaka A (2022) there was a negative correlation between DFI and normal sperm morphology with r = −0.378, p < 0.001. Jakubik-Uljasz J et al. reported that DFI was positively correlated with spermatozoa with head, middle and tail abnormalities, and sperm with cytoplasmic droplets. Sperm DNA fragmentation mainly occurs due to defective maturation of spermatozoa to mature spermatozoa, apoptosis in the testes, and oxidative stress throughout the male reproductive tract.

4.1.2. The relationship between sperm DNA fragmentation with embryo quality, and in vitro fertilization results.

4.1.2.1. Relationship between sperm DNA fragmentation and fertilization results, embryo cleavage results on day 2

Results of fertilization

Our results showed that there was no difference in fertilization rate between the 3 groups of sperm DNA fragmentation degree (p = 0.11),

although in the DFI group, 30% had the lowest fertilization rate. In 3 groups (68.6 6±17.69%). Other studies also recorded low fertilization rates in the high DFI group, but there was no significant difference. The study of Oleszczuk K (2016) reported no influence of DFI on the outcome of insemination in the ICSI technique, although the results of the analysis of fertilization rate in the 0-10% DFI group had the highest value, and this rate gradually decreased in the groups of DFI DFI > 10–20%, DFI > 20–30% and DFI > 30% (p > 0.05).

The study of Xue LT (2016) found that sperm DNA fragmentation rate was negatively correlated with the fertilization rate in ICSI cycles (r=- 0.433, p < 0.001) but not in IVF cycles. Our results also noted that there is a weak negative correlation between the degree of sperm DNA fragmentation and fertilization rate (r = -0.20, p=0.002). Research by author Nguyen Minh Tai Loc also recorded a negative correlation with r = -0.28; p = 0.02. The effect of sperm DNA fragmentation acts early in fertilization, which depends mainly on the quality of the two gametes. Because of this, patients with high levels of sperm DNA fragmentation are unlikely to get pregnant naturally.

Cleavage embryo day 2

In this study, no significant effect of DNA fragmentation on the outcome of day 2 division embryos was observed. However, there was a weak negative correlation between the degree of sperm DNA fragmentation and the ratio of useful embryos on day 2/MII oocytes (r = -0.16, p = 0.01). Author Nguyen Thi Quynh Tien analyzed the correlation between DFI and the results of day 3 division embryos, and found that the rate of day 3 embryogenesis is the ratio between the number of day 3 embryos in the total number of zygotes that were not found. The correlation between embryogenesis on day 3 and DFI index (r = 0.16, p = 0.53), with this ratio we also did not record the correlation (r = 0.01, p = 0.87), but with the calculation of the ratio of embryos to the total number of MII oocytes, we found a weak negative correlation between DFI and the ratio of useful embryos on day 2/MII oocytes (r = - 0.16, p = 0.01).

Sperm DNA fragmentation has been shown to have an impact on mitotic embryos in several studies. In the study of Borges et al. (2019), ICSI results of patients with DFI 30% had a normal division rate and the percentage of good embryos on day 3 decreased statistically significantly compared with the group of DFI <30 %. The results of the study showed that sperm DNA fragmentation did not

affect the embryo division rate when observed under an inverted microscope, but DNA fragmentation did affect the appearance of blastocyst cytoplasmic fragments. In the DFI group ≥30%, the percentage of embryos with no or low-grade cytoplasmic debris was the lowest among the 3 groups, forming the most embryos with cytoplasmic debris in the 3 groups. In addition, an inverse correlation was observed between the degree of sperm DNA fragmentation and the percentage of cytoplasmic fragments <10% (r = -0.17, p = 0.008), and positively correlated with the fraction of cytoplasmic fragments. cytoplasmic rupture > 25% (r=0.18, p=0.006. In 2017, the study by Alvarez Sedo C et al. reported that high DNA fragmentation of sperm samples was associated with the degree of fragmentation cytoplasm was higher than in blastocysts (9.1% vs 15.9%, p < 0.05) program at a higher level than samples with low DFI (16.4% vs 21.9%) (p < 0.05).

4.1.2.2. Relationship between sperm DNA fragmentation and blastocyst

In the study of the author Green KA et al (2020), no difference in fertilization rate, and rate blastocyst /zygote between the two groups of DFI ≤ 15% and DFI > 15% were observed (49.5% vs 48.8%, p = 0.865), besides there was no difference in embryos with polyploidy (55.7% vs 52.1%, p = 0.35). With our study, no significant difference was observed between the three DFI groups, and no correlation was found; however, when evaluating the MII blastocyst/oocyte ratio, although there was no difference, a weak negative correlation was observed with r = - 0.15, p = 0.02.

In addition, our results recorded that the group with the blastocyst/MII <50% had a higher degree of sperm DNA fragmentation than the ≥50% group, the difference tended to be significant. statistics (25.12±14.62 versus 21.71±12.43, p = 0.06). The meta-analysis by Adiga PK et al (2021) also failed to demonstrate any significant difference in blastocyst formation rates between the high and low DFI groups during ICSI. But there was an increase in blastocyst ratio during IVF, the higher incidence in the IVF technique in the high DFI group, this may be because during IVF only spermatozoa had less DNA damage. will be naturally selected, and have better capacity and ability to fertilize and divide to form blastocysts.

4.1.3. Evaluation of the relationship between sperm DNA fragmentation and embryo transfer results

Our results did not have an effect of sperm DNA fragmentation on implantation, gestational sac formation, and fetal progression, although a ratio of clinical values was observed in the DFI group ≥ 30 % was the lowest among the three DFI groups. Two previous studies in Vietnam that performed analysis on day 3 division stage embryo transfer also did not find an association between DFI and clinical outcomes. The effect of DFI on clinical outcomes in ART is currently unknown. Cissen et al analyzed 30 studies to evaluate the value of sperm DNA fragmentation in predicting pregnancy with IVF or ICSI. The authors concluded that current sperm DNA fragmentation assays have a limited ability to predict pregnancy in ART. Results after embryo transfer also depend on many factors of the woman in terms of hormones, endometrial factors ...

4.2 THE IMPACT OF PHYSIOLOGICAL SPERM SELECTION TECHNIQUES ON THE RESULTS OF IN VITRO FERTILIZATION EMBRYOGENESIS.

4.2.1. HBA and relationship with sperm parameters

4.2.2. Results of in vitro fertilization of PICSI and ICSI techniques

Through the results of the study, we found that sperm selection in PICSI did not have more benefits in terms of fertility and embryogenesis rate of day 2 cleavage compared with the ICSI technique. The rate of blastocyst formation was higher in the PICSI group than in the ICSI group, but the difference was not statistically significant: 61.99% versus 58.10%, p = 0.26. This result was also recorded in the study of Majumdar G and Majumdar A (2013): There was no difference in fertilization rate, a good number of embryos between the ICSI and PICSI groups (65.7% vs 64.7%, p = 0.724; 45.8% vs 43.6%, p = 0.460). A previous study using a medium containing hyaluronic acid, evaluating halves of the oocytes by the number of oocytes, did not clearly improve the fertilization and embryogenesis rates compared with ICSI: Choe S.A. et al (2012) reported fertilization rate (75.7% vs 83.0%, p > 0.05), embryo division day 2 rate (72.9% vs 83.0% p) > 0.05), the blastocyst rate on 5/6 was similar in the whole group. However, the percentage of embryos with day 3 division was significantly lower in the PICSI group (56.0% vs 69.6%, p = 0.038).

Research by the authors Liu Y et al (2017): PICSI has a significantly lower rate of abnormal fertilization (1.9% vs 9.7%, p = 0.017) and an increasing trend in fertilization rate. normal sperm count (73.8% vs 62.1%, p = 0.073) with a longer time to perform the technique (2.5 vs 2.1 min, p = 0.001). No difference between PICSI and ICSI was observed in the percentage of good embryos (50% vs 53.1%, p = 0.712). The data reported by the author Kirkman-Brown: the selection of PICSI does not bring advantages in early embryo development, the results of fertilization in PICSI (66.6%) are lower than that of ICSI (69.0%).

There are several reasons for the ineffective results of the sperm selection technique: the technique of collecting sperm from the surface of the PICSI disc at the hyaluronic acid micro point is a mechanical lifting of the adherent sperm from the disc. PICSI leads to a risk of damage to the cytoplasmic membrane at the tip of the sperm, which can affect embryo results. On the other hand, in the process of sperm selection in a PICSI dish and intracytoplasmic sperm injection, the procedure takes longer than conventional ICSI. This can affect the quality of the oocytes when left outside for a long time, so it will reduce the quality of the embryo.

CONCLUSION

Through the study on the influence of sperm DNA fragmentation and sperm selection techniques on the results of in vitro fertilization, we have some conclusions:

1. *The relationship between sperm DNA fragmentation with semen parameters, embryo quality, and in vitro fertilization results.*

1.1. The relationship between sperm DNA fragmentation with semen parameters

- The average level of sperm DNA fragmentation in infertile couples was 23.65 ± 13.80%. The rate of samples with the high levels of DNA fragmentation was 24.4%.

- The semen pH in the DFI ≥ 30% group was the lowest value. DFI has a weak negative correlation with pH with r = - 0.21, p = 0.001.

- The group with low-level DNA fragmentation had progressive motile the highest (p = 0.05).

- Sperm concentration in the DFI ≥ 30% group was the lowest (p = 0.03).

- There was a weak negative correlation r = - 0.13, p = 0.04 between DFI and normal morphology.

1.2. The relationship between sperm DNA fragmentation with embryo quality, and in vitro fertilization results.

- The high DNA fragmentation group had the lowest fertilization rate, the difference was not statistically significant. There was a weak negative correlation between sperm DNA fragmentation and fertilization rate (r = - 0.20, p = 0.02).

- There was no difference in the results of day 2 cleavage embryos between the sperm DNA fragmentation groups. The high DNA fragmentation group showed many blastomeres with > 25% cytoplasmic fragmentation. There was no difference blastocyst generation between the sperm DNA fragmentation groups.

- The rate of pregnancy and ongoing pregnancy in the DFI group ≥ 30% was the lowest among the 3 groups, but the difference was not statistically significant, p > 0.05.

2. *The impact of physiological sperm selection techniques on the results of in vitro fertilization embryogenesis.*

- The difference was not statistically significant in the results of fertilization, and embryo cleavage day 2 between PICSI and ICSI techniques.

- The rate of blastocyst in the PICSI technique tends to be higher than ICSI, but there was no statistical significance. In PICSI, the rate of grade 1 blastocyst formation was lower than that of ICSI, but the rate of grade 2 and 3 blastocyst formation in PICSI was statistically significantly higher than that of ICSI.

RECOMMENDATIONS

- Sperm DNA fragmentation test is a reliable test to assess sperm quality, so it should be applied in parallel with semen analysis in evaluating male reproductive function. Use sperm DNA fragmentation results as a reference value to predict the outcome of in vitro fertilization.

- Reproductive centers need to consider when applying physiological sperm selection techniques.

THE LIST OF WORKS HAS PUBLISHED AND RELATED TO THE THESIS

1. Nguyen Thi Hiep Tuyet, Nguyen Van Trung, Nguyen Thi Thai Thanh, Đang Thi Hong Nhan, Đang Cong Thuan, Le Minh Tam (2021), **Characteristics of sperm DNA fragmentation and the relationship with semen parameters,** *Journal of Medicine and Pharmacy - Hue University of Medicine and Pharmacy*, 5 (11), pp: 103 – 109.
2. Nguyen Thi Hiep Tuyet, Nguyen Van Trung, Nguyen Thi Thai Thanh, Đang Thi Hong Nhan, Le Minh Tam (2021), **Correlation between the degree of sperm DNA fragmentation and the outcome of in vitro fertilization.** Vietnamese Medical Journal, 502 (1), pp:225 – 229.
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5. Nguyen Thi Hiep Tuyet, Nguyen Van Trung, Nguyen Thi Thai Thanh, Đang Thi Hong Nhan, Đang Cong Thuan, Le Minh Tam (2022), **The relationship between the ability to bind hyaluronic acid of sperm with the degree of DNA fragmentation and sperm parameters**, *Journal of Medicine and Pharmacy - Hue University of Medicine and Pharmacy*, 5 (12), tr: 104 – 109.