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Mitofusin 1 as Marker of Oocyte Maturation in Relevance to ICSI Outcome in Infertile Females.

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ABSTRACT

Pro-fusion proteins as mitofusins-1 are required for controlling mitochondrial shape which determined by a dynamic balance between organelle fusion and fission and also supports oocyte development. When compare with somatic cell, mitochondria of oocyte are tiny and circular in presence. The aim is to study the mitofusin-1 in the follicular fluid as a marker for evaluating of oocyte maturation in women undergoing ICSI cycles. Fifty infertile females were included in cross-section study was undergoing ICSI procedure with age 20 to 42 years. After retrieval of oocyte and the number of oocytes was recorded by embryologist and follicular fluid Samples were used for the measurement of Mitofusin 1. Mitofusin 1 levels by ELISA kit (Mybiosource /USA).The results showed considerable higher mitofusin-1levels in patients with good oocytes quality (3.71 ± 1.35 vs. 2.45 ± 1.12 & p=0.001). There were much positive correlations between follicular fluids mitofusin-1 with both total oocytes count (r= 0.374 & p= 0.007) and MII oocytes (r=0.383 & p=0.006); and there was no much correlation between mitofusin-1 levels with MI oocyte (r= - 0.100 & p= 0.490), germinal vesical oocyte (r=0.103 & p= 0.475) and fertilization rate (r= 0.054 & p= 0.711). High follicular fluid levels of Mitofusin 1 may positively impact oocyte development and pregnancy rate.

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KEYWORDS

Phosphatidylserine , Direct Swim Up , Indirect Swim Up , Infertile men.

1. Introduction

Infertility, according to WHO, is an illness of the male or female, defined as failure to conceive after one year or more of regular unprotected sexual intercourse (Vander Borght and Wyns, 2018 [1]). The chance to achieve a pregnancy with one year of unprotected sexual intercourse is 85-90% and 90% within in two years. Difficulties to conceive represented 10-15% of these couples (Abebe et al., 2020 [2]). In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the most commonly used assisted reproductive techniques worldwide. The process of IVF and ICSI needs the harvesting of mature oocytes (Buisman et al., 2020 [3]). Between (10 %_ and 60%) of the oocytes got, after controlled ovarian stimulation (COS) for IVF, were deformed in shape, such as heavy cytoplasm granular, perivitelline pits, refractile body, many vacuoles, thick perivitelline spaces, irregular borders, and a fragment or huge 1st polar body. Deformed shapes may be caused by core influences (like age and chromosomal defect) and/or external influences (like induction protocol, oocyte features, and nutrition) (Chopra et al., 2023 [4]). Follicular fluid delivers the suitable environment for oocyte maturity. It contains hormones with growth factors which involved in ovum maturity and steroid production (Scanlon and Green, 2022 [5]). Pro-proteins such as mitofusin-1 and two control the mitochondrial fusion-fission process. During the development of oocytes and embryos, the effect of mitfusin-1 presents in different stages; an increase and decrease of MEN-1 effects mitochondrial fusion function and ATP metabolism and also arrests oocyte divisions (Zhang et al., 2016 [6]).

2. Patients and Methods

Fifty infertile females were included in a cross-section study was analyzed follicular fluid samples of 50 infertile

women undergo ICSI procedures at the "infertility Center of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies" at Al-Nahrain University at Baghdad/ Iraq for one year. The ages of the 50 women were from 20 to 42 years old, with both types of infertility, primary and secondary. General work-up of infertility was done, like ovulation follow-up, estimation of the tubal patency and uterine cavity, day 2-3 of cycle hormone levels was measured, and seminal fluid assessment was done to all couples. Antagonist protocol for "controlled ovarian hyperstimulation" was used in all patients. Then, the patients had oocyte retrieval, ICSI, and embryo transmission. Pregnancy test done 14 days after embryo transfer.

Inclusion criteria:

- Female aged 22 - 42 years.
- Normal ovarian reserve according to FSH and antral follicle count (AFC) on the second day of the menstrual cycle and AMH.
- Patent and non-patent Fallopian tubes.
- Antagonist protocol.
- Normal or subnormal male seminal fluid analysis.

Exclusion criteria:

✓ Low developmental potential ovarian reserve.

✓ Nonobstructive azoospermia.

✓ Uterine abnormality (congenital and acquired).

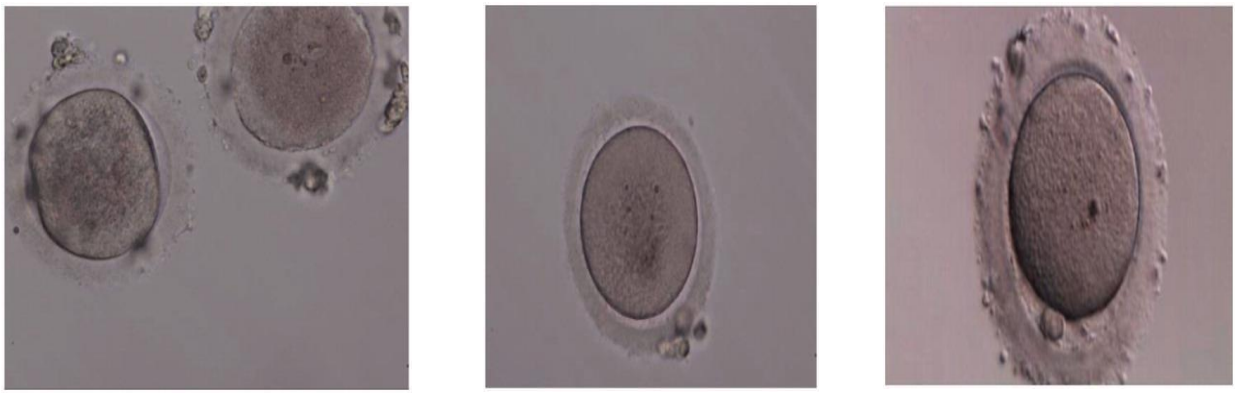
✓ Frozen embryo in cases of low developmental potential endometrium and ovarian hyperstimulation Syndrome.

✓ Patients with endocrine disorders or metabolic disorders.

Follicular fluid Samples were taken during oocyte pickup, which were then centrifuged at 2000g for 10 min, and the supernatants were stored in a deep freezer (-20) for further analysis and to be used for the measurement of Mitofusin 1 level latter on by ELISA kit (MyBioSource /USA)

. After denudation and incubation of oocyte–corona complexes for 2 hrs. After denudation of the oocytes, they were assessed and classified into the following groups (Rienzi et al., 2019 [7]):

- Germinal vesicle that is considered an immature oocyte: its cytoplasm contains central condensed organelles.
- MI (metaphase I) oocyte: there was no germinal vesicle (GV), and the perivitelline space does not contain a polar body (PB).
- MII (A metaphase II) oocyte: single polar body is present, proper perivitelline space, good zona thickness with a normal looking cytoplasm.
- Abnormal oocyte (degenerated): if the cytoplasm was darkened and highly irregular cytoplasm or had condensed smooth endoplasmic reticulum and if the oocytes were too big, with very big PBs, several large vacuoles.



(A) **(B)** **(C)**

Figure 1: Oocytes with different maturation stages (A) GV (B) MI (C) MII.

3. Results:

- **Comparison of follicular fluids mitofusin-1 level in patients with good MII and low developmental potential MII qualities**

The comparison of follicular fluids mitofusin-1 levels between patients with good MII and low developmental potential MII oocyte quality were demonstrated in Table 1 and Figure 2, which showed significantly higher mitofusin-1 in patients with good oocyte quality (3.71 ± 1.35 vs. 2.45 ± 1.12 & *p*=0.001).

Table 1: Comparison of follicular fluids mitofusin-1 levels between patients with good MII and low developmental potential MII oocyte quality

Parameters	Patients with good MII quality N.=26	Patients with low developmental potential MII quality N.=24	*p*-value
F.F. Mitofusin-1 (ng/ml)	3.71 ± 1.35	2.45 ± 1.12	0.001 Ŧ S

SD: Standard deviation; S: Significantly rise (p≤ 0.05) Ŧ: Independent sample t-test

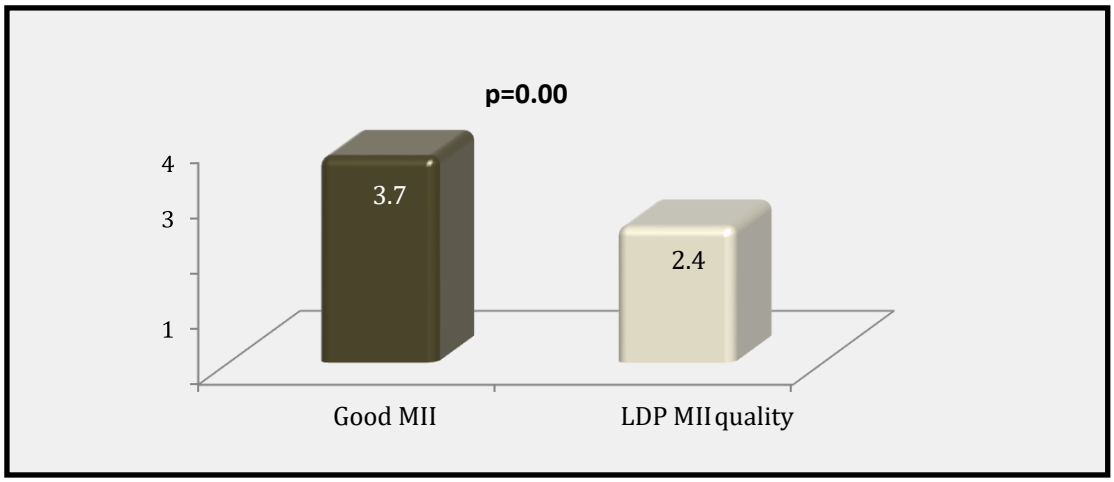


Figure 2: Comparison of follicular fluids mitofusin-1 levels in patients with good MII and low developmental potential (LDP) MII quality.

- **Correlations between follicular fluids mitofusin-1 levels with oocytes ICSI outcome**

  There were important positive relations in follicular fluids mitofusin-1 with both total oocyte count (r = 0.374 & *p* = 0.007), MII oocytes (r = 0.383 & *p* = 0.006), and there was no important relation between mitofusin-1 levels with MI oocyte (r=-0.100 & *p*=0.490), germinal vesical oocyte (r=0.103 & *p*=0.475) and fertilization rate (r=0.054 & *p*=0.711) as demonstrated with table 2, figure (3) and figure (4).

Table 2: Correlations between follicular fluids mitofusin-1 with oocytes -ICSI outcome

Mitofusin-1 correlation with	"Pearson's correlation coefficient" (r)	*p*-value
Oocytes co	0.374	0.007 S
MII Oocyte	0.383	0.006 S
MI	0.100	0.490 NS
Germinal vesicle	0.103	0.475 NS
Fertilization rate	0.054	0.711 NS

MII: Metaphase II; MI: metaphase I; S: Significant ($p \leq 0.05$)

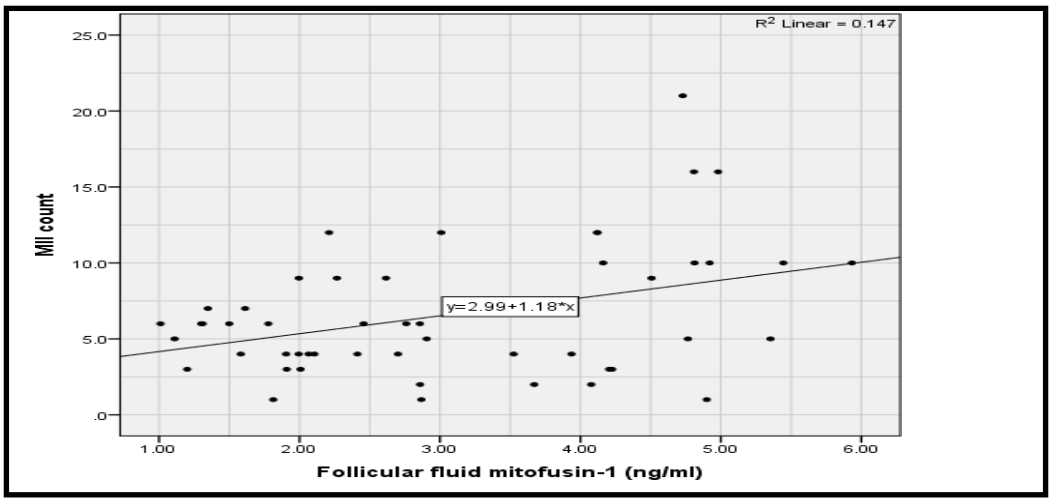


Figure 3: correlation between follicular fluids mitofusin-1 and total oocyte count NS: Not significant ($p>0.05$).

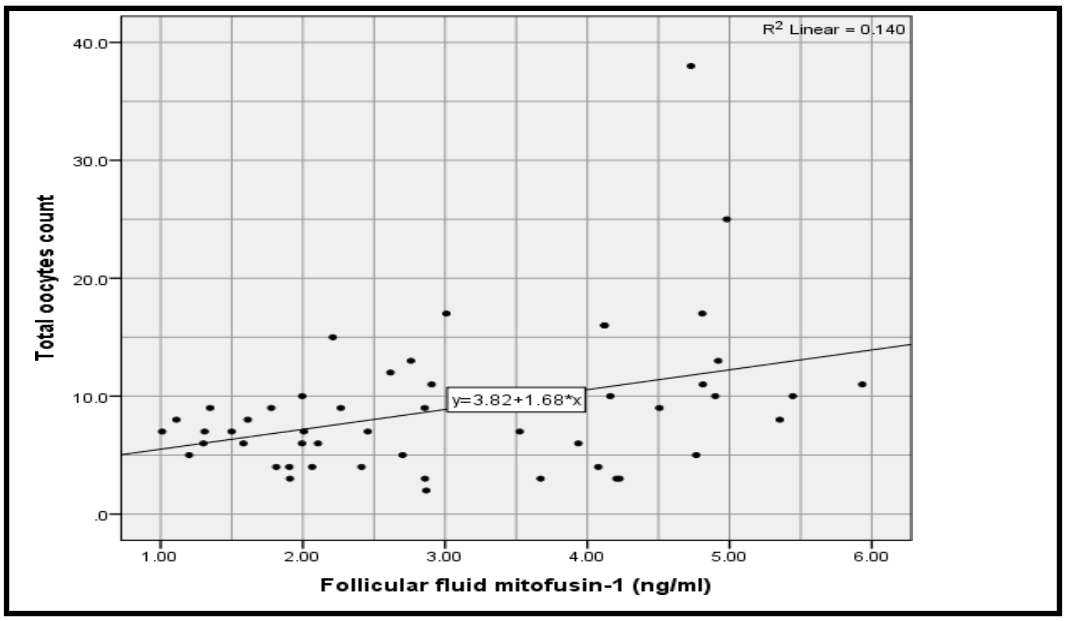


Figure 4: correlation between follicular fluids mitofusin-1 and MII oocyte count

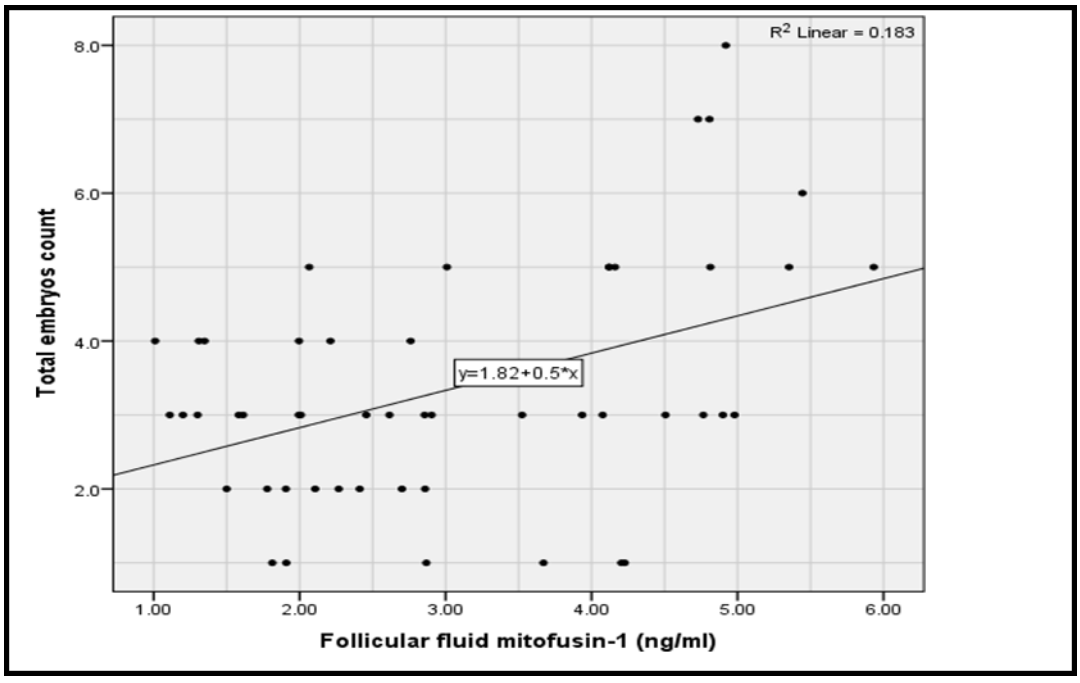


Figure 5: correlation between follicular fluids mitofusin-1 and total embryos count

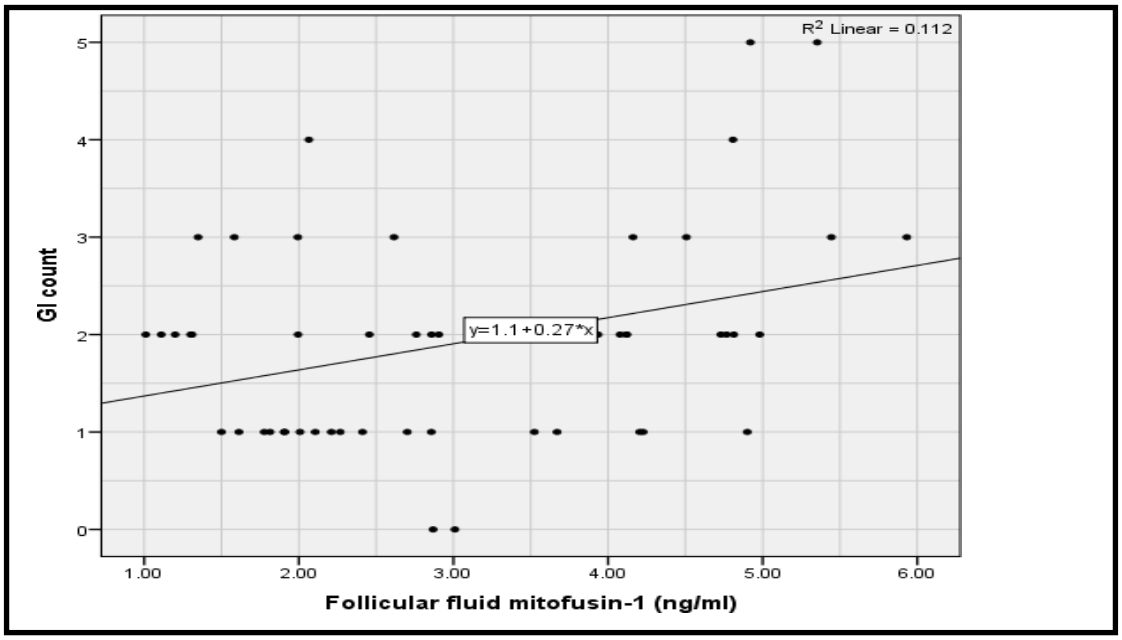


Figure 6: correlation between follicular fluids mitofusin-1 and GI count

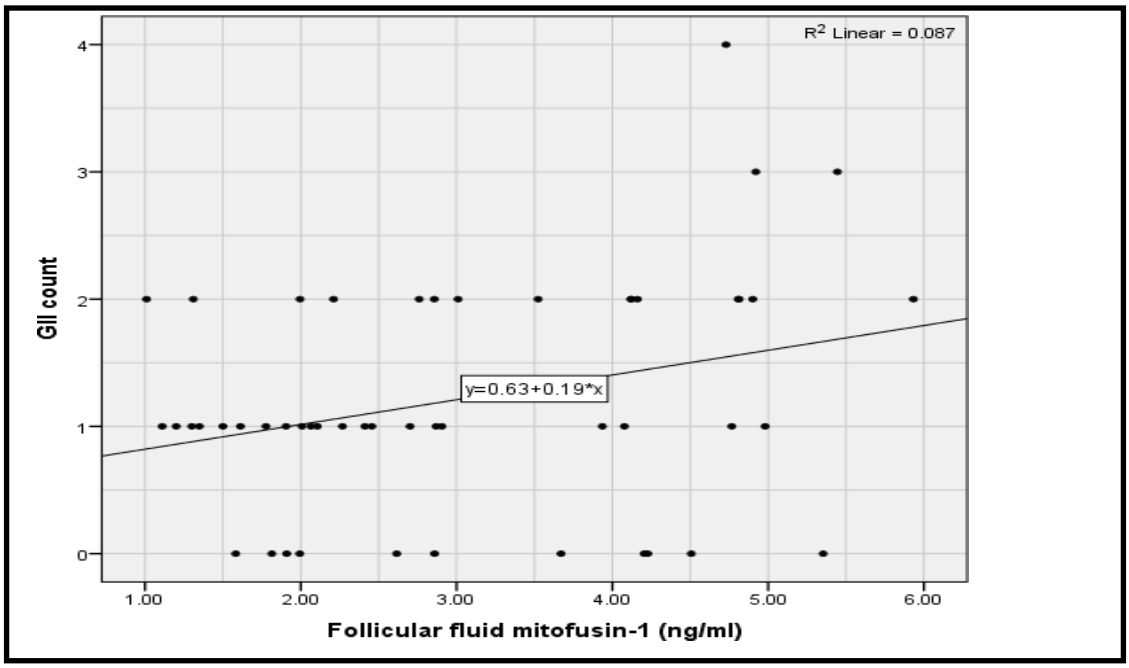


Figure 7: correlation between follicular fluids mitofusin-1 and GII count

4. Discussion

Formation of oocyte and embryo development needs ATP synthesized by mitochondria. The dynamic system of the mitochondria, and especially the fusion of mitochondria, are important for the production of ATP. Fusion is a process that allows mitochondrion to balance the defect in functions by input tRNAs rRNAs with proteins (Udagawa et al., 2014 [8]; Alokbi et al., 2021 [9]). Mitofusin-1 is a main regulator of mitochondrial fusion in mammals. Mitofusin-1 had a main role in the formation of oocyte maturity and embryonic growth, in addition to the risk of Asthenospermia, PCOS, and gestational diabetes mellitus (Zhao et al., 2022 [10]). In a study conducted by Zhang and colleagues, researchers intended to study the role of mitofusin-1 in female reproduction function. They reported that oocyte-specific loss of mitofusin-1 leads to defective follicle growth with diminished of 'ovarian reserve' (Zhang et al., 2019 [6]). In the Zhang et al. study, researchers informed three potential findings regarding mitofusin-1's role on female fertility. First, the oocyte-specific loss of mitofusin-1 results in female sterility as a result of defective oocyte ripening and follicular development. Second, the targeted loss of mitofusin-1 in oocytes causes a defect of adherence and gap junction within oocyte and granulosa cells and increased programmatic cell death. Third, deletion of mitofusin-1 in the ovum causes an accelerated follicular decline (Zhang et al., 2019 [6]).

6- Conclusion

High follicular fluid levels of Mitofusin 1 may positively impact the on-oocyte development and pregnancy rate.

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Author Contribution

Rana R. Khalil performed the study, and Mufeda A. Jwad and Hayder A. L. Mossa supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

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