Effect of follicular fluid vitamin E level on number of oocyte and embryo transferred in ICSI women.

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Abstract
Successful in vitro fertilization outcome requires succession of its primitive elements including good quality and number of both ova and transferred embryo. Therefore, this process is controlled by number of factors that affect these elements, one of the most important factor is the presence of the anti-oxidant substances. Vitamin E is a good anti-oxidant factor that has received a greater attention in recent studies due to its ability to improve reproductive health through its antioxidant action against the reproductive disorders as it serves as a peroxyl radical scavenger that protects polyunsaturated fatty acids (PAFA) in membranes and lipoproteins. The aim of this study is to show the effect of vitamin E level in follicular fluid on the number of oocyte and embryo transferred in patients undergoing IVF/ICSI techniques. In this study follicular fluid vitamin E level were measured at oocyte retrieval day by using Enzyme Linked Immuno-Sorbent Assay (ELISA) test and the number of oocyte were taken, after ICSI process the number of embryo transferred were taken for each women. It was shown that there was a strong positive correlation between follicular fluid VitE level and the number of oocytes (P< 0.008). In contrast no correlation was recorded between its level and the number of embryos.
Keywords: vitamin E; antioxidant; oocyte number; embryo transferred; ICSI techniques.

1. Introduction

1. Infertility

Infertility is a unique medical condition because it involves a couple, rather than a single individual. It is defined as failure of a couple to conceive after 12 months of regular intercourse without use of contraception in women less than 35 years of age and after 6 months in women 35 years and older [1]. While 85% of couples can achieve pregnancy within this interval of time without any assistance, about 15% of couples had difficulty in getting pregnancy [2]. These 15% of couples are considered infertile, which consists of: 35% due to female factors alone, 30% due to male factors alone, 20% due to a combination of female and male factors, and 15% due to unexplained cause of infertility [3]. The initial assessment of infertility is to assess the presence of ovulation, patent fallopian tubes, and normal uterine cavity in women, and to assess normal semenal fluid analysis in men [4]. Infertility has increasingly become a common health problem which has been estimated to affect about 10% of women in their reproductive age, and because of its high prevalence, infertility has been considered as a social disease by the World Health Organization (WHO), its causes are numerous and differ from person to another, and its treatment is of three main therapeutic strategies including: pharmacological therapy, surgical therapy (mostly endoscopy) and assisted reproductive technology (ART) [5].

2. Assisted Reproductive Technology (ART)
ART is a fertility treatment in which both eggs and sperms are handled in the laboratory (i.e., in vitro fertilization or related procedures).Women who undergo ART treatments are much more likely to deliver multiple-birth infants than those who conceive naturally. ART treatment does not guarantee pregnancy and live birth. The success rate is much smaller as compared to the failure rate, it being among its most important limitations [6].
Intracytoplasmic Sperm Injection (ICSI) involves the in vitro injection of preselected spermatozoa in to the cytoplasm of a mature oocyte, after ovarian superovulation, and oocyte retrieval [7]. Currently, ICSI is the most common fertilization technique in the world accounting for about two-thirds of all treatments, while conventional IVF accounts for only around one-third [8]. It is the most effective assisted reproductive procedure and quickly became the favored technique for cases of male factor infertility, as it was discovered that the basic semen parameters, such as having a low sperm count or less motile sperm, had little impact on its success [7, 9]. It is also utilized for the treatment of infertility due to selected female factors including, but not limited to, morphologic anomalies of the oocyte, limited quantities of oocytes, and anomalies of the zona pellucid [10].
3. Vitamin E (α-tocopherol) was firstly discovered by Evans and Bishop in 1922, and it was referred to as an "anti-sterility vitamin" that was necessary to improve reproduction [11]. At that time, it was shown

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that vitamin E might be required in prevention of fetal resorption in pregnancy. Human diet contains 8 different vitamin E-related molecules that was synthesized by plants. These molecules act as a scavenger of peroxyl radical but human body prefers α-tocopherol molecules [12]. Vitamin E has received a greater attention in recent studies due to its ability to improve reproductive health through its antioxidant action against the reproductive disorders [11]. Vitamins can affect the reproductive system through its oxidative mechanism and activity of antioxidants that reduce the excessive production of free radicals. Limited research about the effect of antioxidants on human's fertility are available. However, little studies have been done on the effects of the dietary supplements and multivitamins and antioxidants on both growth and quality of oocytes and embryos. Vitamin E is one of the most important natural antioxidants that protect cells from damage which caused by free radicals. A study which was done by (Ashraf M, Mustansir F, Baqir SM, Alam F, Rehman R,2020) showed that adequate amount of vitamin E in follicular fluid enhances the maturity of oocytes due its anti-oxidant property, which resulted in better reproductive outcome after IVF / ICSI program [13]. There was limited availability of studies regarding the mechanistic functionality of Vitamin E in human reproduction, and so, further detailed human studies are required to evaluate its role in pregnancy.

2. Materials and Methods

This study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology at Al-Nahrain University in Baghdad-Iraq. The duration of the study extended from November 2021 to September 2022.

The study was designed to be prospective study. The sample size was 49 infertile women undergoing intracytoplasmic sperm injection (ICSI) aged between 20-45 years old, their BMI was up to 30 kg/m2. Patients with hypertension, endometriosis or endocrine diseases including diabetes mellitus and thyroid disease were excluded from the study. The apparatus and equipments that were used in the study can be summarized in Table (2.1)

Table (2.1) The apparatus and equipment that were used in the study

Tools and Apparatus	Company/Origin
Automatic Pipette	Slammed, Germany
Centrifuge	Eppendorf, Germany
Enzyme-Linked Immune Sorbent Assay (ELISA)	Sunlong Biotech Co.,Ltd
Eppendorf Tubes	China
Freezer	Concord, Lebanon
Pipette Tips (Yellow)	Brand, Germany
Plain Tubes	AFCO, Jordon

History was taken then examination was done by a specialist doctor in charge at the IVF center .Body mass index was measured by dividing the weight in kilograms by the height in squared meters (kg/m2). Females with BMI of ≤ 18.5 considered as underweight, 18.5-24.9 was normal, 25-29.9 as overweight and ≥ 30 as obese.

Ovulation Induction with GnRH long agonist protocol or GnRH antagonist protocol.

Ovulation trigger when there are 3 or more follicles reach > 17 mm, (Ovitrelle Merck Serono 6500-13000 IU) or (pregnyl Organon 5,000–10,000 IU hCG) were given to induce ovulation and in antagonist protocol sometimes we use 0.2 mg of GnRH agonist.

Ovum pick up

Transvaginal ultrasound guided oocyte retrieval was done 34 -36 hours following the hCG injection under general anesthesia , the induction anesthesia was done by either ketamine or propofol medications. Female was placed in dorsal lithotomy position, vagina cleaned by using copious saline irrigation. Aspiration of the follicles done from ovaries by ovum aspiration needle (Gynetics®, Belgium), suction pressure was 120 mmHg and FF was given immediately to the laboratory, where the assessment and isolation of oocytes can be done.

The number of oocyte was taken and follicular fluid samples were collected for each women enrolled in ICSI procedure at the day of ova pickup. The follicular fluid samples were centrifuged for 15 min at 3000 rpm and a liquid of the supernatants were putting into pyrogen-free tubes and stored at –20°C until assay.

Follicular fluid vitamin E level were measured by using enzyme-linked immune sorbent assay (ELISA), Sunlong Biotech Co., Ltd kit.

ICSI technique

This procedure was done by the clinical embryologist in charge in the Lab. Center. After visualization of the oocyte-cumulus complex (OCC) in the follicular fluid, each oocyte was maintained at 37°C in culture medium with proper pH using 6% CO2 in air through all steps. After 1-2 hour of oocyte retrieval, the denudation was performed by exposure to buffered medium containing 80 IU/ml hyaluronidase to enhance the enzymatic removal of corona cells and cumulus. Then the oocytes were aspirated in and out of a Pasteur pipette and were rinsed several times and incubated for ICSI. The denuded oocytes were examined for nuclear maturation. ICSI was performed 3-5 h after oocyte aspiration by choosing mature MII oocytes which had PB. The procedure was done under a microscope using multiple micromanipulation devices (micro-manipulator, microinjectors and micropipettes).

A holding pipette stabilized the mature oocyte with gentle suction applied by a microinjector. From the opposite side a thin, hollow glass micropipette was

used to collect a single sperm, having immobilized it by cutting its tail with the point of the micropipette. The oocyte was pierced through the oolemma and directed to the inner part of the oocyte (cytoplasm). The sperm was then released into the oocyte. The polar body was positioned at the 12 or 6 o'clock position, to ensure that the inserted micropipette did not disrupt the spindle inside the egg. After the procedure, the oocyte would be placed into cell culture and checked on the following day (after 16-20 hour) for fertilization.

In vitro culture of the embryo

Sequential or single-step culture medium designated for embryo development can be used. In addition, oil overlay used to minimizes the changes in temperature, pH and osmolality. Meanwhile embryo scoring must be performed with evaluation of the cleavage stage of the embryo, according to cell number, size, symmetry, the presence of fragmentation, granulation, in addition to the nuclear status [14]. The embryos grading including: Grade A; blastomeres equal in size without fragmentation, Grade B; blastomere slightly unequal, and up to 10% cytoplasmic fragments, Grade C; blastomeres unequal sized, and up to 50% fragments and large granules, Grade D; blastomeres unequal in size with a significant fragmentation and large black granules.

Embryo transfer

Embryo transfer was performed on day 2 or day 3 post-insemination under transabdominal ultrasound guidance and by using a flexible catheter (Gynetics®, Belgium) that passes through the vagina and the cervix into the uterine cavity, where the embryos were placed under aseptic condition [15]. The selection of embryo for transfer is primarily depending on developmental stage and morphological aspect [16]. The decision on the number of embryos to be transferred must be based on the embryo quality and its developmental stages, as well as female age, ovarian response and the rank of treatment [17]. The number of transferred embryo was taken for each women.

3. Result

This study comprised a total of 49 females who were qualified for IVF process. The demographic and clinical parameters were shown in table (3.1). Around (71.4%) of the participants were within age group 30-39 years, (73.5%) of participants were overweight.

Table (3.1) Demographic and clinical data of studied variables

Demographic and clinical data		No.	%
Age Group/years (32.98 ±4.525)	20-29	9	18.4%
	30-39	35	71.4%
	≥40	5	10.2%
BMI kg/m2 (26.83± 2.533)	Normal (18.5-24.9)	10	20.4%
	Overweight (25-29.9)	36	73.5%
	Obese (≥30)	3	6.1%
No. of oocyte (10.24 ±5.626)	≤10	28	57.1%
	>10	21	42.9%
No. of embryo transferred	1	11	22.4%
	2	12	24.5%
	3	13	26.5%
	4	9	18.4%
	5	4	8.2%

A strong positive correlation was recognized between the number of oocytes and follicular fluid VitE level (P< 0.008). In contrast no correlation was recorded between the number of embryos and VitE level of follicular fluid (fig. 1 & 2 respectively).

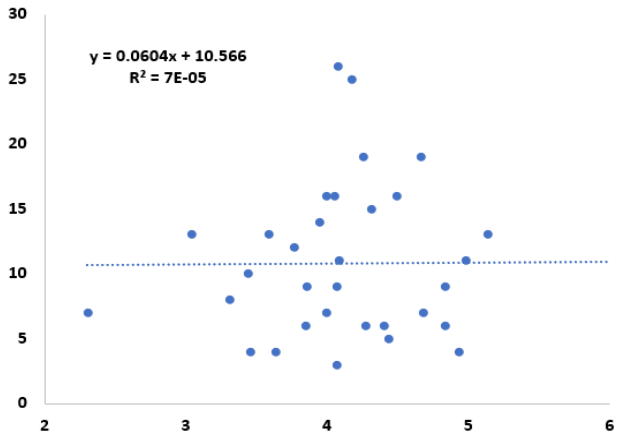

Fig (3.4): Correlation between no. of oocytes and Vit E level of follicullar fluid P< 0.008

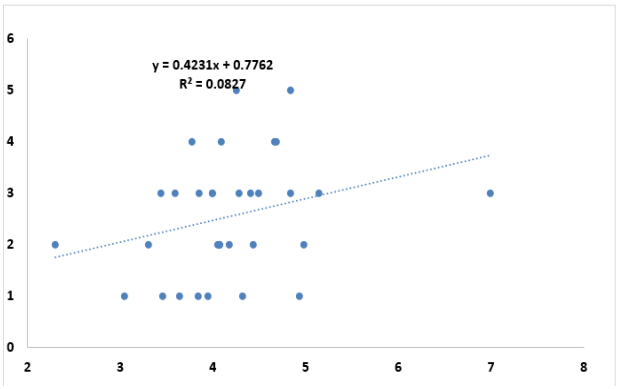

Fig (3.5): Correlation between no. of embryos and follicular fluid vit E. P value not significant.

4. Discussion

Vitamin E level and oocytes number

In the present study, we determined the level of vitamin E in follicular fluid and investigated their relationships with oocytes number and quality as a

clinical parameter in IVF outcome. Generally, our findings did not reveal any significant correlation between Vit E level oocytes number, so far when do further analysis of our data, a fascinating significant correlation was found between Vit E follicular fluid and number of oocyte (P<0.008) as long as this level was below 5 ug\ml., these findings are in support with Bahadori and his team results. They specified that It seems that at a certain levels of vitamin E we can have higher maturated oocytes [18]. Furthermore, various previous studies were agreed to some extent with the current study for instance Tareq and his colleagues who suggested that Vit-E increased oocyte maturation and fertilization by reducing the accumulation of ammonia and toxic subtances [19]. Likewise, Ashraf etal proposed that adequate amount of VE in follicular fluid improves the opportunity of maturation of oocytes on account of the anti-oxidant potential, which resulted in better IVF outcome [13].

Vitamin E level and embryos number.

A study carried out in women undergoing IVF showed that, vitamin E present in the follicular fluid, is related to higher oocyte maturation and higher quality embryos rates [20, 21]. What's more, no significant correlation was reported between the number of embryos and follicular vit E level which is nearly concomitant with Bahadori etal conclusions even though he investigated the quality but not the number of embryos [18].

5. Conclusion

Follicular fluid vitamin E level (when it was below 5 ug) is a good predictor for number of oocyte but it does not correlate to the number of embryos transferred.

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