Effects of T-Consciousness Fields on Mouse Oocyte Maturation and Embryo Development Following IVF

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Abstract

T-Consciousness Fields (TCFs) with non-material and non-energetic entities have been introduced by Taheri. The influence of TCFs can be investigated through laboratory experiments. In this study, two separate works were conducted to evaluate the effects of TCFs on the *in vitro* maturation of mouse oocytes (IVM), mitochondrial membrane potential and embryo development through *in vitro* fertilization (IVF). In the first experiment, after 24 hours, the maturation of oocytes either in the presence or absence of TCFs was observed using an inverted microscope and the mitochondrial membrane potential of MII oocytes was estimated through JC-1-aggregate fluorescence intensity. In the second experiment, the effects of TCFs on the rate of fertilization and 2PN zygotes and the grades of embryos were evaluated. The results showed that the maturation of oocytes under TCFs treatment increased by an average of 33% compared to the control (p-value<0.05), and the MII oocytes had a higher mitochondrial membrane potential under the influence of TCF1 (p-value<0.05) and TCF3 (p-value<0.01), suggesting their better efficiency. Moreover, TCFs increased the probability of 2PN zygotes by about 12% compared to the control (p-value=0.0090). Furthermore, TCFs-treated samples exhibited a notable 45% reduction in grade BC (p-value<0.05). Additionally, the percentage of grades A and B of oocytes was more than two times (p-value <0.05) higher than the control. In conclusion, these results provide preliminary evidence of the positive effects of TCFs treatment on fertilization and pave the way for further research in this area.

Keywords: Consciousness; Oocyte; embryo; mouse; maturation; fertilization

Introduction

Infertility is a medical condition defined by the inability to achieve a clinical pregnancy despite 12 months of consistent and unprotected sexual activity. It is estimated that approximately 8 to 12% of couples are suffering from this issue all around the world (Ombelet, 2020; Vander Borght and Wyns, 2018). Generally, aside from unexplained reasons that it affects 15% of couples and about 85% of infertility cases arise from ovulatory dysfunction, male factor infertility, and tubal disease (Ameratunga et al., 2023; Carson and Kallen, 2021; Guideline Group on Unexplained Infertility et al., 2023; Teede et al., 2023). Additionally, lifestyle and environmental factors, such as smoking and obesity, can have a detrimental effect on fertility as well as endocrine disturbances (Ameratunga et al., 2023; Bala et al. 2021; Emokpae and Brown 202; Chai et al., 2023; Linehan et al., 2022; Malekpour et al., 2023; Tzeng et al., 2023).

Assisted reproductive technology (ART) has facilitated the treatment of infertility over the past three decades, resulting in the birth of millions of children worldwide (Glujovsky et al., 2023; Ono et al., 2023; Pinborg et al., 2023; Sachs-Guedj et al., 2023). Although *in vitro* fertilization (IVF) with ovarian hyperstimulation is an appropriate option for many couples, this treatment may not be suitable for some patients such as those women with high antral follicle count (AFC) and polycystic ovary syndrome (PCOS) (Das and Son, 2023; Meneghini et al., 2023; Sachs-Guedj et al., 2023; Wesevich et al., 2023;Thakre and Homburg, 2019). In these cases, IVM is an alternative way, which involves the process of oocyte maturation *in vitro* without prior gonadotrophin stimulation (Das and Son, 2023; Zhang et al., 2023; Coticchio, 2016).

Consciousness is described as an elusive phenomenon, and it has been stated that no scientific theory can truly explain it (Markkula, 2015; Polák and Marvan, 2018; Schurger and Graziano, 2022). The most popular explanations about consciousness have been presented by neuroscience, such as global neuronal workspace (Luczak and Kubo, 2021; Owen et al., 2023; Rabuffo et al., 2022; Mashour et al., 2020), integrated information theory (Albantakis et al., 2023; Barbosa et al., 2021; Tononi et al., 2016) and higher-order thought theory (Arsiwalla et al., 2023; Ludwig, 2022; Revach and Salti, 2022). Historically, while dualism describes consciousness as a non-physical entity separate from the physical body, physicalism views it as an integral part of the material world. Additionally, panpsychism posits that consciousness is inherent in all things (Hoffman et al., 2023; Sanfey, 2023; Goff, 2017).

In the 1980s, Mohammad Ali Taheri introduced consciousness as a fundamental element of the universe from which information, matter and energy spring forth. In this context, we refer to this form of consciousness as 'T-Consciousness' to differentiate it from other conventional theories. According to this perspective, there are various T-Consciousness Fields (TCFs) with different functions, which are the subcategories of a networked universal internet called the Cosmic Consciousness Network (CCN) (Taheri, 2013). One of the unique aspects of this theory is its applicable features. These fields can be examined through reproducible experiments. Indeed, it is possible to apply TCFs to all living organisms and inanimate materials, providing a brilliant opportunity to understand consciousness as a non-physical entity. According to Taheri, transmitted information via TCFs may lead to alterations in the behavior of treated samples in comparison with controls (without TCFs treatment). To our knowledge, this is the first time that consciousness can be investigated through laboratory tests (Torabi et al., 2020).

The practical application of TCFs has motivated us to investigate their effects on different subjects. Not only do these studies provide a valuable opportunity to understand the notion of consciousness, but they also enable us to evaluate whether information can be transmitted through TCFs. Previously, they have been applied to different subjects ranging from plants (Torabi et al., 2020) and cell line (Taheri

et al., 2022a) to materials (Taheri et al., 2021). In this study, two experiments were separately conducted to investigate the effects of TCFs on the *in vitro* maturation of mouse oocytes (IVM), mitochondrial membrane potential, and embryo development through *in vitro* fertilization (IVF). These tests can provide evidence of information transmission via TCFs and evaluate the potential influence of this treatment on embryo development.

Material and Methods

TCFs application

The samples were subjected to TCFs treatment according to protocols regulated by the COSMOintel research center (www.cosmointel.com) and the resources provided by the innovator of the theory (Taheri et al., 2022). In this research, TCFs were applied at the time of the beginning of each experiment to the named plates of immature oocytes (in the IVM experiment) and mature oocyte and sperm system (in the IVF experiment).

Chemicals and animals for IVM experiment

All chemicals were purchased from Sigma (St Louis, MO, USA) except follitropin alfa (Gonal-F), recombinant human follicle stimulating hormone (rhFSH), and human chorionic gonadotropin (hCG) which were purchased from Organon (Oss, Netherlands), and fetal calf serum (FCS) which was purchased from Invitrogen (Carlsbad, CA, USA).

The experiment was performed using 6–8 weeks-old female NMRI mice with an approximate weight of 20-25 grams. They were purchased from Royan Research Institute in Tehran, Iran. The mice were housed under a controlled environment of 22 ± 3 °C temperature and 12 hours of light/dark cycle.

All animal experiments were carried out according to the guidelines of the Iranian Council for Use and Care of Animals and approved by the Animal Research Ethical Committee of Tehran University of Medical Sciences (Ethical Committee code: IR. TUMS. VCR. REC.1399.5341).

In vitro maturation of oocytes

Healthy female mice were sacrificed via cervical dislocation and their ovaries were extracted under sterile conditions. After that, they were freed from all connective tissues and placed in 100 µl of α-minimal essential medium (MEM) supplemented with 5 mg/ml streptomycin, 6 mg/ml penicillin, 5% fetal calf serum (FCS, Invitrogen, USA), 100 mIU/ml recombinant human follicle stimulating hormone (rhFSH), and 7.5 IU/ml human chorionic gonadotropin (hCG, Sigma, USA) under mineral oil. Antral follicles were released from the ovary using an insulin syringe, and granulosa cells surrounding them were removed by pipetting. Immature oocytes (GV) with uniform zona pellucida, translucent cytoplasm and suitable perivitelline space were collected using a stereo microscope (Nikon SMZ- 2T, Japan) and kept in 20-µl drops of culture for 24 hours to IVM.

About 300 separated immature oocytes were divided randomly into five groups. In the first group, immature oocytes were placed in a culture medium containing MEM-α, 75 mIU/ml rFSH, 7.5 IU HCG and FCS 5%. In the second group, immature oocytes were put in the mentioned culture medium with inducing granulosa cells (Positive control). Three types of TCFs, including TCF1, TCF2 and TCF3 were applied to three other experimental groups, respectively according to the TCFs application explanation section. Each experimental group was cultured in a humid incubator at 37 °C with 5% CO2 (Memmert, Schwabach, Germany). The characterization of the oocytes was evaluated after 24 hours by the use of an inverted microscope (Labamed, USA). The classification of oocytes was as follows: GV when the germinal vesicle (GV) was identifiable, GVBD when the GV was not present, and MII when the first polar body was extruded according to Nikseresht, 2015.

Mitochondrial membrane potential

Mitochondrial membrane potential was measured in this study according to the Cossarizza et al. 1996 study. JC-1 dye can serve as an indicator of mitochondrial potential in diverse cell types. The green to red fluorescence ratio relies solely on the mitochondrial membrane potential and is not affected by factors like mitochondrial size, shape, and density. By employing fluorescence ratio detection, researchers are able to make relative measurements of membrane potential and ascertain the proportion of mitochondria within a group that reacts to a given stimulus (Sivandzade et al., 2019).

To assess mitochondrial membrane potential (MMP) and stain MII oocytes, 1 µL of JC-1 dye (red) was mixed with MEM-α culture medium containing 49 µL of serum and incubated for 30 minutes in 50 µL droplets, with two mature oocytes in each drop, in the dark. The samples were then washed with serum-containing medium while avoiding light exposure. The oocytes were examined using a Nikon fluorescence microscope. Fluorescence intensity was analyzed using ImageJ software. The average MMP of the oocytes was calculated as the ratio of red fluorescence intensity to green fluorescence intensity.

Experimental design for IVF

Animals

In this study, 6–8-week-old NMRI male mice were used as sperm donors (n=5). Also, 6-8 weeks old NMRI mice were used for egg donation (n=28). Mice were kept according to the standard protocol, including 12 hours of light and 12 hours of darkness, and finally, they were killed by cervical dislocation.

Embryo culture medium

This study used a KSOM culture medium containing 0.1 g of BSA was used. (Fraction V; Sigma, Cat. # A9647; lot # 15H0672) according to Erbach *et al* (1994).

Sperm preparation

Fertile male mice were killed and the tail of the epididymis was immediately transferred to a 1500 µl drop of culture medium covered with mineral oil (Sigma: embryo-tested, Cat. #M8410). The contents of the epididymis were removed and the remaining tail tissue was discarded. The petri dish containing sperm was kept in an incubator for one hour at 37 °C with 5% CO2 in humid air for the capacitation process according to Henkel (2012).

Adult egg collection

Ovulation stimulation of female mice was done by the intraperitoneally (i.p) injection of 7.5 international units of mare serum gonadotropin (PMSG) and 48 hours later, 7.5 i.u. of human chorionic gonadotropin (HCG) were injected according to Helmy *et al*. 2023. In the next step, the eggs were collected 14 hours after HCG injection by removing the oviduct of the female mouse and placing them in the culture medium at 37°C. Cumulus–oocyte complex (COC) retrieved from antral follicles was collected by an insulin needle. Then, the cumulus oocyte masses are pitted until the eggs become single, and finally, we put every 3-5 ovules in drops of 50 µl of the culture medium, which are placed under mineral oil.

In vitro fertilization

IVF was performed as described by Tokuhiro *et al* (2012). It was performed in 50 µl drops of KSOM under mineral oil. A pre-incubated sperm suspension for the capacitation process was slowly added to the collected oocytes to obtain a final motile sperm concentration of $1x10^6$/ml. The combined sperm-oocyte suspension was incubated for 4-6 hours. The fertilization rate

was evaluated by recording the number of 2-cell embryos 24 hours after *in vitro* fertilization completion.

Statistical analysis

Experiments were repeated at least three times. We didn't exclude any samples from the analysis. For all tests, experimenters were blinded to treatment status. All data are presented as mean ± standard deviation (SD) followed by one-way analysis of variance and multiple comparisons with a 95% confidence interval, and significant p-values less than 0.05. All analyses were carried out with GraphPad Prism version 9.

Results

IVM under the influence of TCFs

The meiotic stages of the oocyte were examined after 24 hours of *in vitro* maturation (Table 1, Figure 1). The average number of immature oocytes reduced by approximately 20% in the positive control, compared to the negative control. Additionally, this reduction occurred by around 33%, 20%, and 36% for TCF1, TCF2, and TCF3, (p-value<0.05) respectively. Moreover, an enhancement in MII oocytes can be observed in TCF1 and TCF3 experimental groups, exhibiting an average increase of approximately 40% (p-value<0.01) compared to the negative control. In the last column of this table, the sum of the MII and GVBD exhibited a noticeable change under the influence of these three types of TCFs (p-value<0.05).

Table 1. The changes in meiotic stages for *in vitro*-cultured mouse oocytes

Experimental Groups*	GV No. %±SD	GVBD No. %±SD	MII No. %±SD	MII+GVBD %±SD
Control (-)	43±9	20±0	37±9	57±9
Control (+)	23±9	17±5	60±8	77±9
TCF1	10±8a	13±5	77±12b	90±8a
TCF2	13±9a	29±9	58±6	87±9a
TCF3	7±9a	16±4	78±6b	93±9a

* Initial oocyte No. in all groups was 60. GV: germinal vesicle, GVBD: germinal vesicle breakdown, MII: metaphase II, TCFs: Taheri Consciousness Fields. All values shown by mean ± standard deviation a: difference with the negative control p-value<0.05, and b: p-value<0.01.

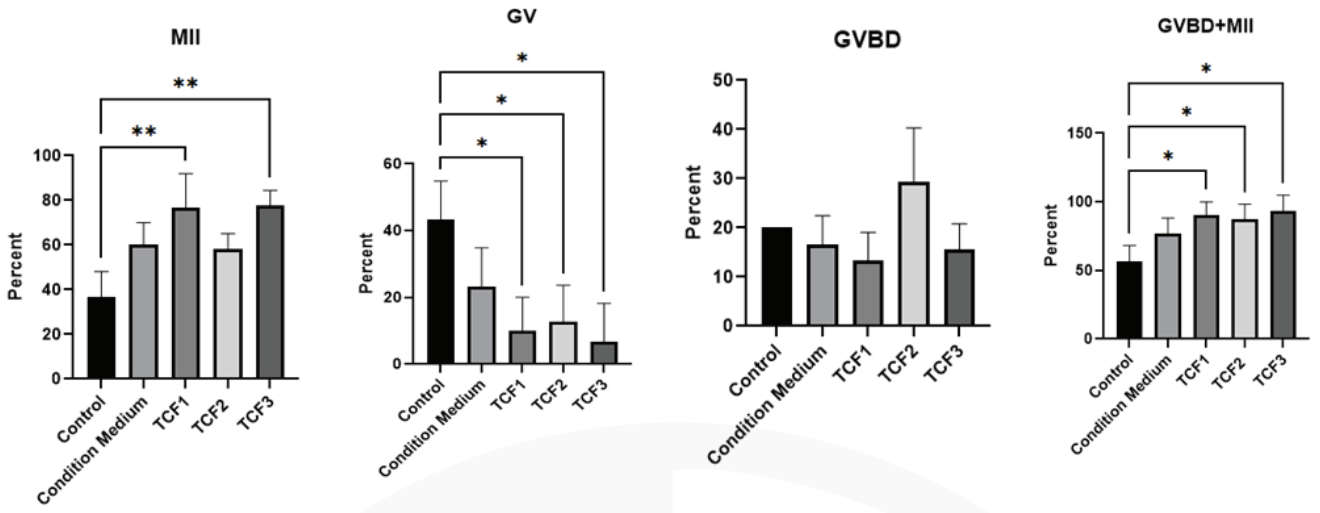
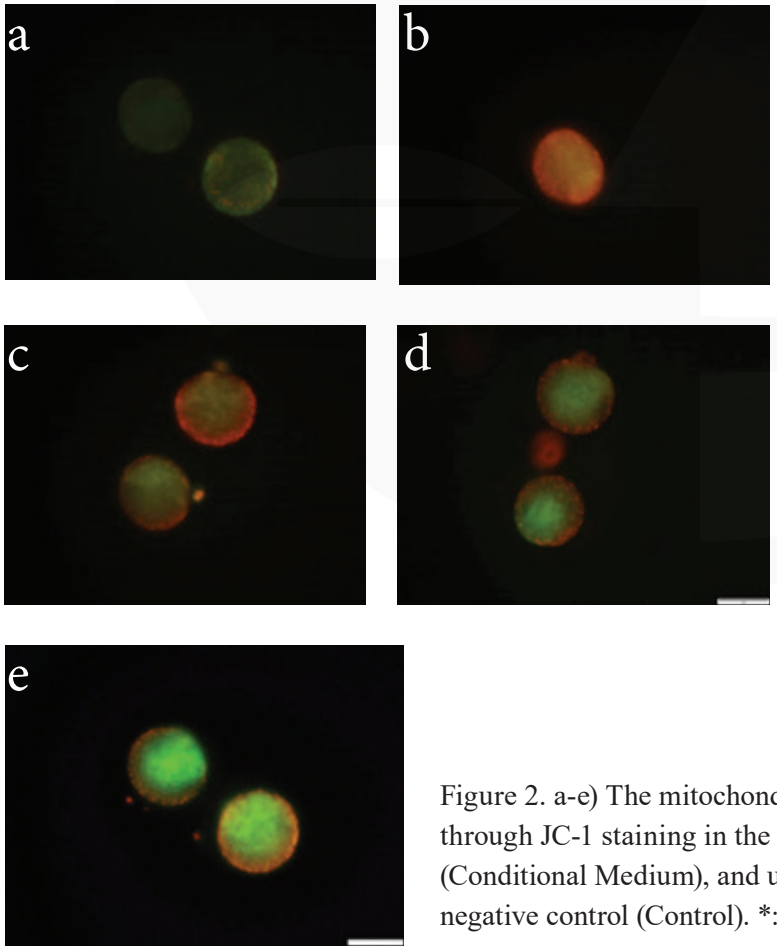


Figure 1. Comparison of oocytes at various stages of meiosis in different sample and control groups, including Germinal Vesicle (GV), Germinal Vesicle Breakdown (GVBD), and Metaphase II (MII). *: p-value<0.05, **: p-value<0.01.

Figure 2 indicates the mitochondrial membrane potential in the experimental groups. The value of the condition medium (positive control) was significantly higher than that of the negative control, by 82% (Fig 2, e). Similarly, in comparison with negative control, a noticeable increase of approximately 61% and 69% can be observed for the samples under TCF1 and TCF3, respectively.



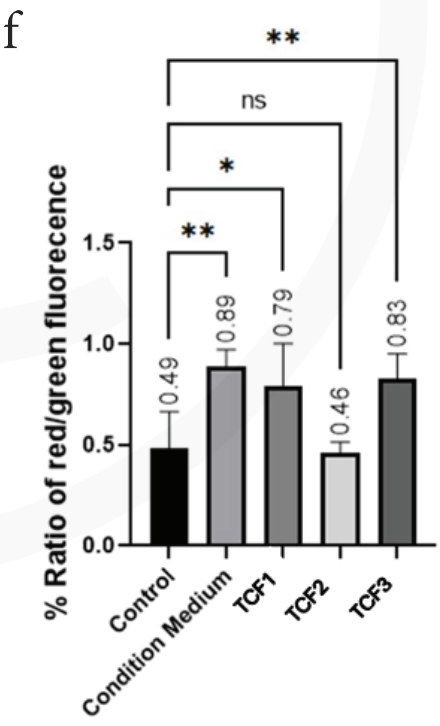


Figure 2. a-e) The mitochondrial membrane potential (ΔΨm) was visualized through JC-1 staining in the MII stage. f) ΔΨm values in the control positive (Conditional Medium), and under the influence of TCF1, TCF2, TCF3, and the negative control (Control). *: p-value<0.05, **: p-value<0.01.

IVF under the influence of TCFs

TCFs increased the percentage of 2PN zygotes by approximately 12% and reduced unfertilized oocytes by 10% compared to the control (Table 2, Fig. 3). However, in other states, the changes were not statistically significant under TCFs treatment. Additionally, Two-cell formation rate was almost similar in both experimental groups (Table 3).

Table 2. *In vitro* fertilization outcomes in TCFs-treated samples and controls.

	Control	Control (%)	Sample	Sample (%)	Delta
2 PN Zygote	120	58.5	145	70.7	12.2
Immature oocyte	5	2.4	7	3.4	1.0
Oocyte	56	27.3	36	17.6	-9.8
Parthenogenesis	12	5.9	11	5.4	-0.5
Degenerated oocyte	11	5.4	5	2.4	-2.9
2-cell	1	0.5	1	0.5	0.0
Total	205		205		

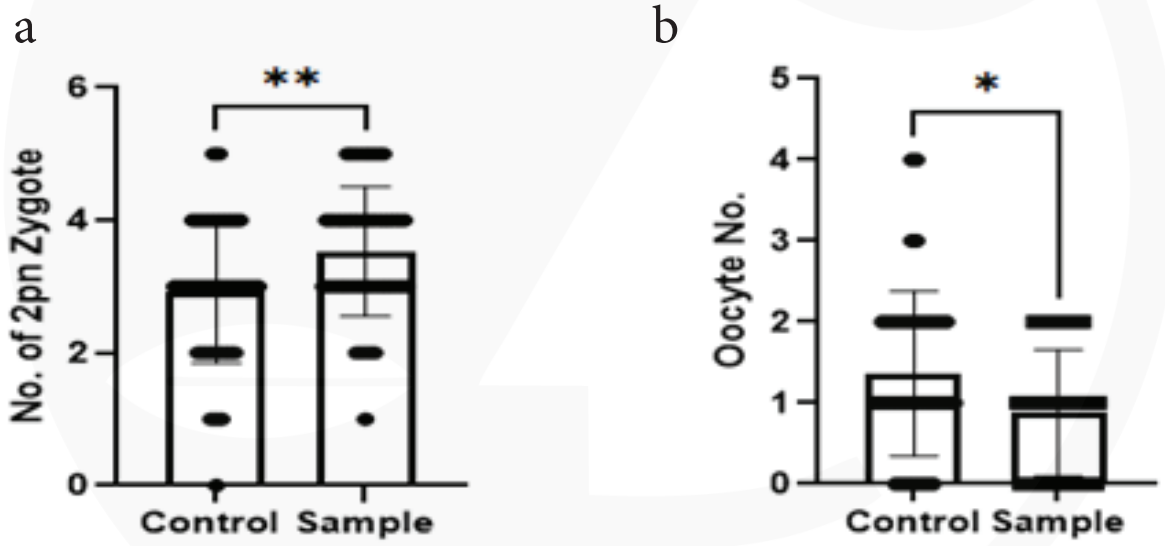


Figure 3. The number of 2PN zygotes (a) and unfertilized oocytes (b) in samples under TCFs treatment and control. **: p-value=0.0090, *: p-value=0.0172.

The changes of zygotes in experimental groups

After 24 hours, the changes in obtained zygotes presented in the previous section have also been illustrated in Table 3. Although there were no significant differences (p-value=0.07) between TCFs-treated samples compared to the control, an increasing trend in the rate or 2-cell zygotes can be observed under this treatment. Indeed, the distribution of values showed an alteration as a result of exposing TCFs.

Table 3. The rate of two-cell and other states of the zygotes in control and samples under TCFs treatment.

	Control	Control (%)	Sample	Sample (%)	Δ% (S-C)
2 Cell	65	53.7	79	54.1	0.4
2PN	54	44.6	64	43.8	-0.8
Polyspermy	1	0.8	0	0.0	-0.8
Parthenogenesis	1	0.8	1	0.7	-0.1
Degenerated Embryo	0	0.0	2	1.4	1.4
SUM	121		146		

Evaluation of embryo grade

The grades of embryos in the control and TCFs-treated samples have been presented in Table 4. The percentage of A and B grades under TCFs was approximately two times higher than that of the control group (p-value<0.05). Furthermore, there was a reduction of about 45% in the BC grade, as low-quality embryos, under the influence of TCFs (p-value<0.05).

Table 4. The percentage of different grades of embryos in control and samples under TCFs treatment. *: difference with the control group p-value< 0.05.

	Control	Control (%)	TCFs	TCFs (%)	Δ% (TCFs-Control)
A	3	4.6	11*	13.9	9.3
AB	32	49.2	38	48.1	-1.1
B	5	7.7	12*	15.2	7.5
BC	18	27.7	12*	15.2	-12.5
C	7	10.8	6	7.6	-3.2
SUM	65		79		

Discussion

In this study for the first time the effect of TCFs, as a treatment with non-physical entities, on IVM and IVF was investigated. The initial phase of the current experiment demonstrated that TCFs treatment resulted in an improvement in oocyte maturation, characterized by a higher mitochondrial membrane potential. This observation was in line with our previous studies. It has been found that a cell line had a better viability under TCFs treatment (Taheri et al., 2022a). Moreover, the effect of TCFs on ATP production in the HEK-293 cell line was investigated, and the results showed a significant increase in ATP concentration in samples treated with TCFs compared to the control group (Taheri et al., 2022). It is well documented that there is a definite link between oocyte developmental competence and mitochondrial function (Wu et al., 2015). A number of studies have suggested that defects in mitochondrial membrane potential can lead to a reduction in ATP production, oocyte maturation, and embryo development (Al-Zubaidi et al., 2019; Grindler and Moley, 2013).

To further explore the effects of TCF treatment, an IVF experiment was designed as the second part of this study. The obtained data showed that not only did this treatment enhance the probability of 2PN zygotes, but it also increased the embryo quality. Generally, embryos have been graded based on the degree of cellular fragmentation and the regularity of blastomere size. Accordingly, embryo quality is described

as grade A, even-sized blastomeres with no/minimal fragmentation; grade B, few uneven-sized cell with minor fragmentation; grade C, uneven-sized cells with moderate fragmentation; grade D, mostly uneven-sized cells with sever fragmentation; grad E, few blastomere of any size with severe fragmentation according to previous literature (Helmy et al., 2023; Li et al., 2015). In the present test, a higher percentage of grade A and B were observed under these non-physical treatments, suggesting an improved quality as a consequence of exposure to TCFs.

As it has been described in the introduction section, Taheri's theory posits that consciousness plays an important role in our frequency universe through which matter and energy originated from it. Historically, the description of consciousness has primarily focused on humans since the 17th century (Seager, 2007, p. 9), and still there is not a universally accepted definition about this most profound of mysteries. Indeed, we cannot accept the existence of consciousness and its influence by solely reading a pile of books; rather, it is necessary to experience it through conducting laboratory experiments. The applicable feature of TCFs allows us to design diverse scientific studies, offering empirical evidence of their influence.

In the realm of physics, the concept of 'Field' has been extensively employed, encompassing familiar laws like gravitational and electromagnetic fields. Now, novel fields have been introduced by Taheri, involving non-physical entities that can have detectable impacts on various subjects. Though it may appear unconventional to investigate the influence of a non-observable phenomena, it is quite common in the history of science. For instance, in cosmology, the most well-known example is dark energy, discovered through observing its effect on universe's expansion (S. Turner and Huterer, 2007). The same can be said for exploring the existence of these fields with non-observable features. Therefore, in the current study regardless of knowing the mechanism of TCFs, and how they may change the behavior of the samples, the first step is recording their influence. This enables us to detect the interaction between consciousness with matter and energy.

According to Taheri, samples exposed to TCFs receive information and consequently exhibit behavior that differs from that of the control (Taheri et al., 2022b). One of the first models of information theory is the communication model introduced by Shannon and Weaver (1949). The communication theory assumes that before receiving some information the system is in a physical state characterized by maximum uncertainty and maximum entropy. Upon receiving the information, entropy decreases (Hoffman et al., 2023; Shannon, 2001). To clarify this concept, the changes in entropy for the TCFs-treated samples and control groups in the current study will be calculated and explained in a subsequent paper.

While the impact of physical fields such as static magnetic field on the mice oocytes has been reported by other researchers (Baniasadi et al., 2023), the peasant results are considered as the first investigation of the effects of TCFs on IVF and IVM studies. Although the exact mechanism of the effect of these novel fields remains unknown, the research finding confirms the positive effects of TCFs treatment. Additional studies are required to further molecular studies about the effectiveness of these novel fields. Moreover, investigating the effects of TCFs on intracytoplasmic sperm injection (ICSI) as well as the *in vivo* development of the mouse embryo beyond the blastocyst stage within the uterine environment is on the agenda of the authors of this study. Collecting additional evidence can assist in elucidating the potential advantages of TCFs treatment.

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