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Comparison of the results of *in vitro* fertility cycles between cryopreservation method by vitrification oocytes and fresh oocytes in human

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Abstract

Oocyte cryopreservation addresses several difficulties, such as maintaining female fertility, oocyte accumulation in women with decreased ovarian reserve, inadequate ovarian stimulation response, or developed "oocyte donation bank". A retrospective and prospective cohort study were conducted at the Infertility Department - Hanh Phuc International Hospital, recording the results of 210 couples undergoing IVF cycles, with the goal of comparing and evaluating the effectiveness of treatment in two groups using frozen-thawed oocytes and fresh oocytes in IVF cycles (105 cycles using thawed oocytes and 105 cycles using fresh oocytes). Intracytoplasmic sperm injection was conducted on all mature oocytes from fresh and thawed oocytes (frozen and thawed by vitrification technique) (ICSI). The embryological and clinical results of the two groups were documented, compared, and validated using the t-test. Survival rate of the freezing - thawing process oocytes was 92.61% and there was no difference in the embryological and clinical outcome results between thawed and fresh oocytes, through the following criteria: the rate of fertilized oocytes (69.04% vs 73.43%, p = 0.1026) with good embryo (29.10% vs 33.87%, p = 0.1794), the rate of blastocyst formation (45.61% vs 56.55%, p = 0.0541). The cumulative pregnancy rate, clinical pregnancy rate, live birth rate was 47.17%, 40.75% and 35.51%, respectively, while group using thawed oocytes was 40.79%, 50.03% and 34.76%, respectively (p = 0.3176 – 0.2377 – 0.9067). The miscarriage rates in the two groups were 6.04% and 5.51% (p = 0.7615).

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Introduction

After decades of study, *in vitro* fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) technologies have been developed in the field of assisted reproduction procedures to allow infertile couples to have a chance to have children. Patients assigned to ICSI for infertility treatment have poor sperm quality (low numbers, abnormally shaped sperm, and a low motile sperm count), sperms were obtained surgically, directly from the testicle/ epididymis, and previous IVF treatments were unsuccessful (Palermo *et al.*, 1992; Devroey and Steirteghem, 2004; Palermo *et al.*, 2009). ICSI is especially useful for insemination with cryopreserved oocytes since freezing can promote premature exocytosis of the ovule granules and hardening of the transparent membrane (zona pellucida), preventing natural sperm penetration. ICSI is the optimal insemination technique for avoiding repeated fertilization, insemination with high oocyte counts, and producing the greatest number of embryos (Chian *et al.*, 2014; Dayal *et al.*, 2014).

Frozen cell samples were thought to be unattainable in the early 1900s. Polge, on the other hand, discovered in 1950 that glycerol could preserve cow sperm through freezing and thawing. Since then, with the discovery of dimethyl-sulfoxide (DMSO) better freezing protection, cases of effective oocyte cryopreservation in rats, rats, hamsters, rabbits, and primates have been reported on a regular basis (Whittingham, 1977; Chian *et al.*, 2014). In 1986, the first successful human oocyte cryopreservation was reported by Chen, with an oocyte survival rate of 80% and a fertilization rate of 83%; 6 - 8 cells stage embryo development was 60% (Speroff *et al.*, 2014). However, at the time, oocyte cryopreservation was still mostly done by slow freezing, with varying success rates (ranging from 0 to 60%), which had a substantial impact on oocyte quality, fertilization, and embryo development. The emergence of the vitrification technology in the 1990s was a watershed moment in helping to solve the challenges mentioned above (Porco, 2018). This technique is based on high-speed cooling by direct immersion in liquid nitrogen, reducing cell damage because no ice crystal formation occurs. There have been many reports in the world proving that oocyte cryopreservation and embryo cryopreservation by vitrification technique have superior efficiency compared to the slow freezing technique (Oktay *et al.*, 2006; Cobo *et al.*, 2009; Glujovsky *et al.*, 2014). In Vietnam, cryopreservation of human oocytes has been introduced into the treatment of infertility since 2002, and the first clinical pregnancy case was recorded in September 2003 from oocyte cryopreservation. The first clinical pregnancy from oocyte cryopreservation via vitrification was recorded in 2006. According to recorded data, approximately six babies were born from oocyte cryopreservation between 2006 and 2010, with a total of over 200 estimated to date (Vinh *et al.*, 2011). Along with the advancement of assisted reproductive techniques in Vietnam, cryopreservation of human oocytes is becoming more popular for social reasons, particularly in cases of oocyte donation or freezing.

In recent studies by Huyen *et al.*, 2018 and Kha *et al.*, 2020, there are intuitive results and evaluation of the efficacy of the vitrification method when studied on 211 mature oocyte cryopreservation (corresponding to 34 cycles of oocyte cryopreservation and 34 cycles of embryo transfer) at the Army Clinical Embryology Institute, Academy Military medicine (Huyen *et al.*, 2018) and at multi-centers of the IVFMD system from the following thawing, oocytes will be cultured and inseminated using ICSI techniques as usual (Han *et al.*, 2020). Both studies found that the oocyte freezing method can be used to help preserve human oocytes in patients undergoing IVF.

Currently, three types of instruments are commonly used in vitrification stages: the High-Security Vitrification Kit (HSV), cryotip, and cryotop. These are kits that make it simple and convenient to load samples, fix samples, seal samples when placed in thermometers, immerse samples in liquid nitrogen bottles, and move samples to perform the freezing process. Because each kit has different benefits and drawbacks, fertility clinics in Vietnam are now using

vitrification techniques to freeze human oocytes. Depending on the actual situation at each center, the storage and defrosting process and the storage tools and equipment used can be adjusted to suit the operation method of the center.

Although oocyte cryopreservation by vitrification is presently commonly employed in Vietnam, no research comparing the therapeutic benefits of vitrification and fresh oocytes has been conducted. As a result, further investigations are required to enhance scientific knowledge in this field. This is the scientific foundation upon which specialized doctors counsel people while treating "infertility" to get the best possible outcome. The primary goal of this study was to assess the efficiency of the vitrification method of freezing and thawing human oocytes at the Assisted Reproductive laboratory, Infertility Department - Hanh Phuc International Hospital's IVF room.

Materials and methods

Types of studies

This is a retrospective and prospective cohort study from 210 cycles of *in vitro* fertilization at the Infertility Department - Hanh Phuc International Hospital from January 2015 to December 2021.

The use of patient data was allowed by Hanh Phuc International Hospital's Scientific and Ethical Committee under code 80a/BVHP-HDYD. The study was divided into two groups group one (Vitrification): oocytes maintained through vitrification and group two (Fresh): fresh oocytes.

Types of participants

Patients participating in this study were 21-49 years old, negative for HIV and infectious diseases, and sperm samples of relatively normal quality according to WHO 2021 standards with semen volume ≥ 1.4 ml, sperm concentration ≥ 16 million sperm/ml, total sperm count ≥ 39 million sperm, motile sperm ratio ≥ 30%, sperm morphology ≥ 2%. Mature oocytes at Metaphase II (MII oocytes) showed no abnormalities in morphology, cytoplasm and cell membranes.

Sperm preparation

After abstaining from intercourse for 2-7 days, semen was collected by masturbation; then, sperm samples were processed by the swim-up method used for ICSI (WHO, 2010).

Oocyte preparation

Patients were ovarian stimulated according to the protocol at the Infertility Department - Hanh Phuc International Hospital by injecting Diphereline 3.75 mg for folliculogenesis in 10-12 days, periodically checked by ultrasound transducer and their estradiol and progesterone levels were tested on a regular basis to monitor follicular development. When there were at least two follicles of 14 mm in size, the follicular fluid containing the oocytes was collected into a specialized tube to segregate the collected oocytes (Speroff *et al*., 2014; Eshre, 2019; Elder and Dale, 2020).

Oocyte cryopreservation by vitrification technique

The vitrification oocyte procedure was used with cryotec and preserved in liquid nitrogen at -196^{o}C according to the instructions included with the Cryotech kit. The plate of a 4-well was numbered from 1 to 3, with well 1 having 300 µL of equilibration solution (ES) and wells 2 and 3 containing 300 µL of vitrification solution (VS). Oocytes were placed on the surface of ES medium in well 1 for 15 min at laboratory temperature to equilibrate in the medium. Transfer the oocytes with the least amount of ES medium to two wells containing VS medium, wash the oocytes for 30-40 seconds, and repeat the washing of the oocytes in wells 2 and 3 for 10-20 seconds. Transfer the oocytes in extremely small amounts of VS medium onto the cryotec and immediately submerge the cryotec in liquid nitrogen produced in a styrofoam container (390 x 290 x 335 cm and a depth of 20 cm). Then use a clamp to close the cryotec cap, transfer to cryocane and then transfer to the MVE sample flask.

Thawing oocytes

The thawing oocyte procedure followed the Cryotech kit instructions by ultrafast high-temperature heating.

Two plates of a 4-well were used, plate 1 contained 500 cc of warming solution (TS), which was maintained heated at 37°C, plate 2 was marked with wells 1, 2, 3, with well 1 holding 300 µL of diluent solution (DS), wells 2 and 3 containing 300 µL of washing solution (WS), plate 2 is stabilized at room temperature. The frozen oocyte was placed in liquid nitrogen, the cryocane and cryotec cap were removed, the cryotec was dipped into TS medium to locate oocytes, and the oocytes were held in the medium for 1 minute. Transfer oocytes with about 3 mm lengths of TS medium to wells containing DS medium, wash and soak for 3 minutes in the medium. Continue to transfer oocytes with about 3 mm lengths of DS medium to wells containing WS medium, wash, and soak for 5 minutes in the medium. Oocytes were moved to well 4 of the WS medium and washed twice for 1 minute each time. Finally, before ICSI, the oocytes were transferred to culture media for oocyte recovery. The survival rate of post-frozen oocytes was determined by examining the morphology under a stereo microscope at 40X magnification. Mature and live oocytes morphology was oocytes that have a bright, homogeneous cell complex, a clearly visible first polar body, an oval shape, and a smooth surface which contain all of the components required for *in vitro* fertilization and have no effect on the freezing process by vitrification technique. The periovulatory cavity had an irregular shape that was fractured and granular, whereas thawed oocytes had dark, uneven cell complexes and the first polar body could not be recognized to determine its dead form (WHO, 2010; Alpha, 2011; Arav *et al.*, 2019).

Insemination by intracytoplasmic sperm injection (ICSI)

The technique of ICSI is performed on the ICSI micromanipulator system by WHO, 2010 with the temperature must always kept consistent at 37°C and the time to execute ICSI is as short as feasible. Transfer the oocytes to a culture dish after ICSI, each with a 20 µL microdroplet of distinct, independent CSCC-NX media covered with 2 ml of mineral oil in the culture dish. The culture plate will be put in a separate culture chamber in the culture cabinet (37°C, CO_2 6%, O_2 5%), labelled with information for each case, fertilization rate and embryo growth evaluation, and embryo quality.

Fertilization rate and embryo development and quality

After 17 hours (± 1 hour) of ICSI, the oocytes were removed from the culture machine to evaluate the fertilization morphology, assess the quality of embryos on day 2-3 at 48-68 hours (± 1 hour) and evaluate blastocyst quality at 116 hours (± 1 hour). The criteria for evaluating the fertilization phenomena and determining the quality of the 3rd day embryos and blastocysts are consistent with the Alpha Consensus criteria for assessing and classifying embryo quality. Accordingly, the fertilization rate was determined with 2 polar bodies and 2 pronuclei with separate membranes, of equal size, located close to each other in the central region of the ovum.

The embryo morphology on 2nd or 3rd day corresponds to embryos with 4-8 cells, divided into 4 types including type I embryos (good type) with 8 cells and fragmentation rate <10%, associated blastomeres well, none of the blastocysts were multinucleated; type II embryos (fair type) with 8-cell embryos, fragmentation rate of 10-20%, weak linkages, no multinucleated blastomeres; type III embryos (medium type) with embryos of 6,7 or 8 cells, fragmentation rate 20% or even blastomeres, weak linkages, no multinucleated blastomeres; type IV embryos (poor grade) with embryos with more than 8 cells, or 4-6 cells, or 8 cells with more than 20% fragments or unequal blastomeres or with multinucleated blastomeres (Alpha, 2011).

The blastocyst was evaluated according to the Alpha association, including 3 groups as good, fair, and moderate. The blastocyst, when observed under the microscope, will be evaluated for growth rate with embryo morphology in early blastocysts, blastocyst maturity stage, expanded blastocyst, or blastocyst that is or has ruptured. The endoplasmic reticulum and stromal cell layer are factors for classifying blastocyst types (Alper *et al.*, 2018).

Embryo storage, thawing and embryo transfer

Embryos were frozen at either the 3rd day (6 – 8 cells) or 5th day (blastocyst) stage, depending on the patient's request. Embryos were vitrified and kept in liquid nitrogen flasks. When a patient wanted to transfer embryos, the embryos were withdrawn from the storage vessel and defrosted in the same way that oocytes were before being put in a concentric dish for embryo transfer. The embryo transfer plate was withdrawn from the culture cabinet when the signal to transfer embryos was given. Under microscopic manipulation, embryos were put into the embryo transfer catheter and then transferred into the patient's uterus by a doctor. Under the microscope, checked for any remaining embryos after transfer.

Determining embryo transfer results

14 days after the embryo transfer, the patient will have blood taken to test the hCG level. An hCG level greater than 50 mIU/mL suggests chemical pregnancy. The fetus will continue to grow in the mother's uterus until the fifth week when clinical indicators of pregnancy arise when an ultrasound shows the gestational sac (which can be conducted as early as 4-5 weeks after the period). If gestational sac imaging is not accessible, biochemical pregnancy will be documented. The clinical number of fetuses would be reported once the pregnancy and heartbeat had been detected. The number of fetuses that advanced was recorded when the fetus continued to develop until the infant was born. However, if the pregnancy stops growing at any point after 7 weeks, it will be recorded as a miscarriage.

Statistical analysis

GraphPad Prism version 9.3.1 was used to calculate statistical processing based on the unpaired t-test to evaluate the difference between the experiments group. P <.05 was considered statistically significant with 95% confidence. The results were presented as the Means ± SE (Standard Error).

Results

Cycles of in vitro fertilization characteristics

Age is closely related to the quantity and quality of oocytes in female patients, after the age of 35, the number of follicles is only about 10% of what it was at puberty, and the quality of the follicles also drops dramatically (Vinh *et al.*, 2011). Meanwhile, BMI will examine potential health hazards; based on the scale of the Asia-Pacific Association, the normal index is 18.5 - 22.9. AMH is a hormone released by ovarian follicular cells that does not vary much during the menstrual cycle and can properly determine a woman's ovarian fertility with normal serum levels ranging from 2 to 6.8 ng/ml (Dayal, 2013). The results of the cycles of *in vitro* fertilization characteristics are shown in Table 1.

Table 1. Cycles of *in vitro* fertilization characteristics.

Group	Sample size	Age			BMI	AMH	Oocyte collected
		Min	Max	Average			
Vitrification	N = 105	25	49	31.52 ± 5.56	22.79 ± 3.13	2.95 ± 2.15	1350
Fresh	N = 105	21	46	31.80 ± 5.06	23.10 ± 3.59	3.22 ± 2.12	1837

N: number of cases.

N: number of cases

There was no significant difference between the two groups of patients using the vitrification oocyte and the patients using fresh eggs, ensuring the results that the study required (p-values of age, BMI, AMH and total oocytes obtained were 0.4644; 0.5068; 0.3519; 0.4369, respectively). This study found that the baseline characteristics of the two patient groups in this study were consistent. This contributes to the study's following outcomes being objective and very accurate.

The survival rate of oocytes after thawing by vitrification oocytes

The number of thawed oocytes after freezing was 1337 oocytes, of which 1245 oocytes were alive after thawing (92.61 ± 10.41%) and 92 oocytes were degraded (7.39 ± 10.41%).

Table 2. Differences in fetal development and birth from vitrification versus fresh oocytes**.**

Oder Number	Orders	Vitrification		Fresh		p-value
		N	Rate (%)	N	Rate (%)	
1	Number of patients	105		105		
2	Number of embryo transfer cycles	138		152		
3	Number of embryo transfer	292		304		
4	Average embryo	2.15		2.02		
5	Number of embryos transfer day 3	219	75.00	138	45.59	
6	Number of embryos transfer day 5	73	25.00	166	54.61	
7	Chemical pregnancy rate	11	6.43	15	8.95	0.4415
8	Nesting rate	63	26.11	52	24.58	0.7306
9	Cumulative pregnancy rate	70	47.17	55	40.79	0.3176
10	Clinical pregnancy	52	40.75	65	50.03	0.2377
11	Prolonged pregnancy rate	46	35.98	43	34.76	0.8473
12	Live birth rate	43	35.51	43	34.76	0.9067
13	Miscarriage rate	7	6.04	5	5.51	0.7615

N: number of cases.

The number of patients over 35 years old with a 100% survival rate after thawing was 12 cases (52.17%) (Fig. 1).

Effect of in vitro fertilization between vitrification oocytes and fresh oocytes

The total number of normally fertilized oocytes obtained from vitrification and fresh oocytes was 873 and 1105 oocytes, respectively, with normal fertilization rates of 69.04 ± 19.42% and 73.43 ± 16.65% ($p > 0.05$). The average number of fertilized oocytes in a patient with vitrification oocytes was 8.31 ± 5.80 oocytes, and 10.52 ± 5.84 oocytes in a patient with fresh oocytes. The overall number of normally fertilized oocytes in patients over the age of 35 with frozen oocytes was 153, whereas fresh oocytes were 203.

The embryo formation rate after ICSI was 95.21 ± 10.59% for vitrification oocytes and 97.38 ± 10.88% for fresh oocytes, respectively. The normal fertilization rate and embryo formation rate were not significantly different between the two study groups, with p-values of 0.1026 and 0.2603, respectively (Fig. 2). The embryos accumulated on the 2nd or 3rd day would be consistent with the overall cell shape, blastocyst count, blastocyst uniformity, and fragmentation rate (Fig. 3). The rate of embryo formation in the vitrification oocyte group was 29.10 ± 26.85%; 41.14 ± 25.53%; 30.59 ± 30.73%; and 4.71 ± 12.66%. For the fresh oocyte group, the rate of embryo formation in the fresh oocyte group was 33.87 ± 24.39%; 39.63 ± 24.80%; 23.68 ± 22.52%; 2.27 ± 7.63%, respectively (294; 464; 220 and 22 embryos). Similarly, to the results for all types of the embryo in the frozen oocyte group, type II embryos had the largest number following embryo division, with poor embryos recording for just a very small section (5%). Statistical comparison of each type of embryo between frozen and fresh oocytes showed that there was no difference in the ratio with P of 0.1794; 0.6635; 0.0645; 0.0928, respectively. Therefore, frozen oocytes by vitrification technique did not affect the rate of formation of 3rd-day embryos.

After the 3rd day embryos were identified and cultivated, and the patient should make a selection about the embryos' status. The 3rd day embryos will either continue to freeze or develop to become the 5th day embryos, with experimental interventions following. There were 35 patients who continued to cultivate embryos on the 3rd day to become blastocysts in the frozen oocyte group and 63 patients in the fresh oocyte group. In both groups, the average number of blastocyst-cultured embryos per patient was 10.57 and 10.65, respectively.

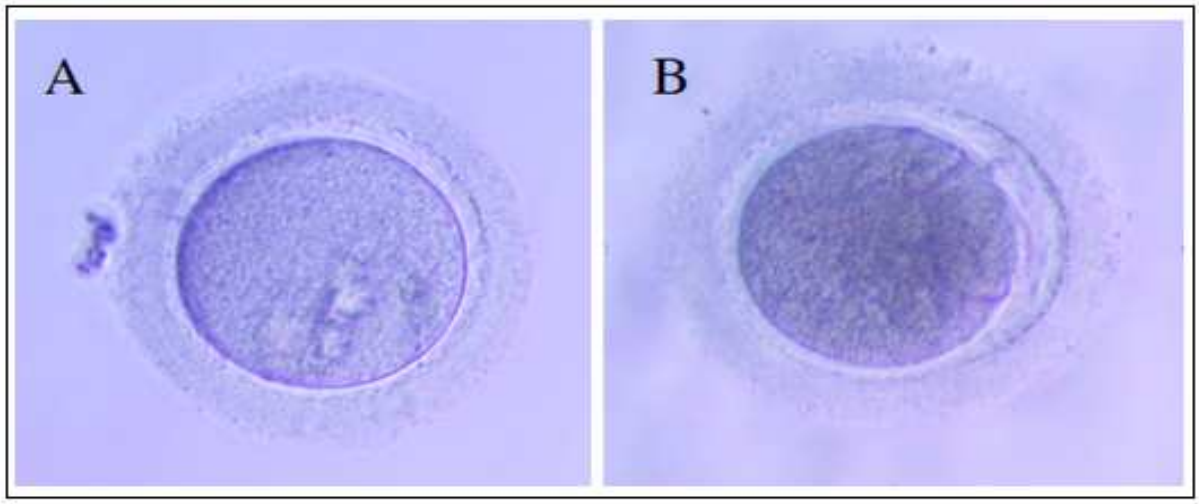

Fig. 1. Morphology of thawed oocytes with normal morphology (A) and degraded morphology (B).

In both groups, the average number of blastocysts cultivated was 5.29 and 6.17 embryos, respectively. The blastocyst culture rate and quantity of blastocysts did not differ among groups, with P values of 0.0541; 0.7834; 0.1374 and 0.1111, respectively. Type II embryos accounted for a significant portion of all embryos. As a result, the vitrification procedure had no effect on the rate of blastocyst formation on the 5th day.

The results of embryo transfer and in fetal development to birth between vitrification and fresh oocytes

The results of embryo transfer and the number of cycles of embryo transfer are shown in Table 2. There was no statistically significant difference in the number of embryo transfers or the number of embryos transferred (p = 0.1663 and 0.6064, respectively.

The number of embryos that opted to transfer on 3rd or 5th day differed dramatically. In the frozen oocyte group, embryos would be prioritized for transfer on the 3rd day with 219 (75%) embryos in the oocyte group. On the 5th day, 166 fresh embryos (54.61%) would be transplanted. The clinical outcomes were similarly comparable; in the group using fresh oocytes, the cumulative pregnancy rate, clinical pregnancy rate, and live birth rate were 47.17%, 40.75%, and 35.51%, respectively, whereas in the group utilizing thawed oocytes, they were 40.79%, 50.03%, and 35.51%, respectively. The miscarriage rate in the two groups was 6.04% and 5.51% (p = 0.7615). The study also recorded that oocyte freezing by vitrification method helps to reduce the rate of oocyte degeneration due to the impact of the freezing-thawing process, with the survival rate of oocytes after thawing reaching 92.61%.

Discussion

Vitrification is a technological evolution in assisted reproduction since it allows infertile couples who donate late to have healthy babies and children. The survival rate and normal morphology of oocytes after thawing by vitrification technique were 89.09.% (Huyen *et al.*, 2018) with 118 live oocytes out of 211 thawed oocytes; 99.4% (Monica *et al.*, 2007) with 328 out of 330 oocytes; 95.59% (Kha *et al.*, 2020).

Because cells undergoing freezing and thawing processes will endure changes affecting this morphology, characterized by membrane shrinkage, membrane dissociation, and translocation of membrane proteins, when the cell membrane is affected, the important component of the cell is genetic material, there will be changes in the structure and function (Ahn *et al.*, 2002), which leads to genetic material breakage, especially in reproductive cells such as ovules and spermatozoa with single chromosome sets that are easily susceptible to damage by temperature (Best BP *et al.*, 2015).

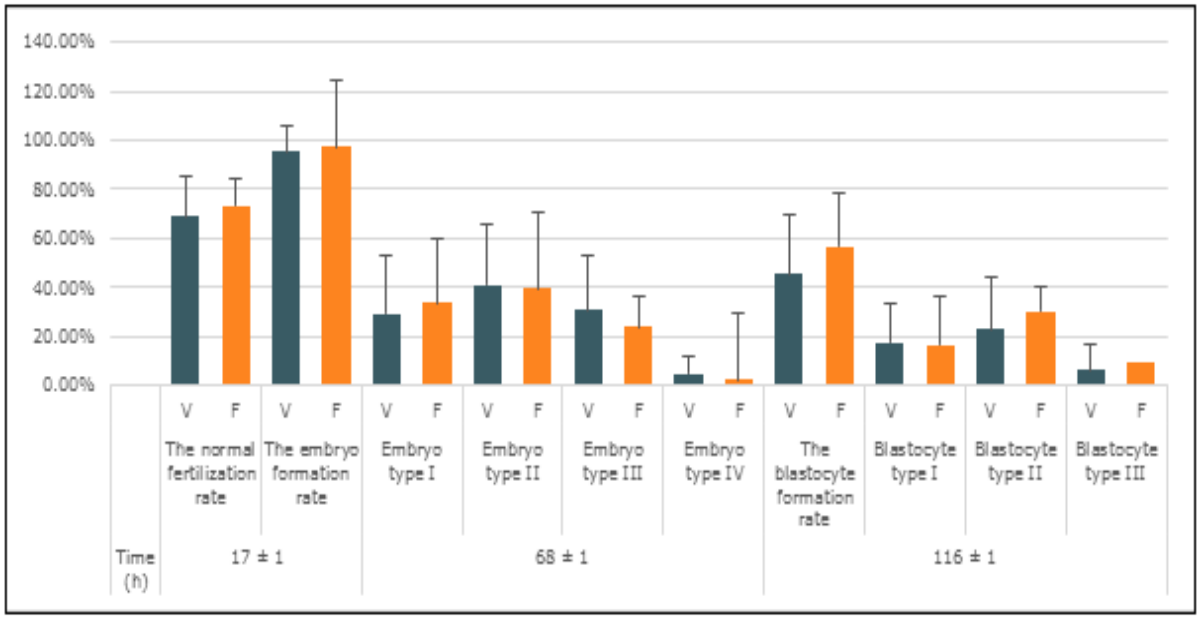

Fig. 2. Effect of in vitro fertilization between vitrification and fresh oocytes.

In a study by Huyen *et al.*, 2018, with vitrification oocytes, the good embryo number on 3rd day was 48/124 (38.71%); the average number of embryos transferred was 2.5; in 34 embryo transfer cycles, 12 cycles of biochemical pregnancy (35.29%), 10 cycles of clinical pregnancy (29.41%), of which 9 cases of one pregnancy, 1 case of two pregnancies, and 1 case of miscarriage (8, 3%) at 12 weeks; nesting rate: 11/85 (12.94%). Similarly, in Monica *et al.*, 2006's study, 295 embryos (6-8 cells) were transplanted into 120 patients (2.45 embryos/transfer) with 39 individuals had a positive hCG test 14 days following embryo transfer; pregnancy was identified after 5 weeks, yielding a pregnancy rate of 32.5% and an implantation rate per embryo of 13.2%. As a result, eight of the 39 patients (20.5%) experienced spontaneous abortions, while the other 28 had prolonged pregnancies.

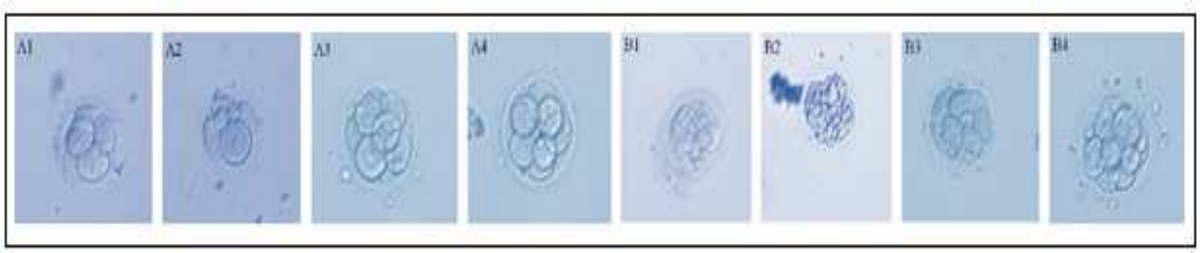

Fig. 3. Morphology of oocytes on 2nd or 3rd day between vitrification oocytes (A1 - A4) and fresh oocytes (B1 - B4). A1: 2nd day embryo type I; A2: 2nd day embryo type II; A3: 3rd day embryo type I; A4: 3rd day embryo type II). B1: 3rd day embryo type III; B2: 2nd day embryo type IV.

Three healthy infants have been born at the time of writing (in October 2005, January 2006, and June 2006). According to Cobo *et al.*, 2011, the vitrification oocytes group had a 40.0% embryo transfer rate, a 49.1% chemical pregnancy rate, and a 55.4% clinical pregnancy rate. Finally, in recent research by Kha *et al.*, 2020 from June 2017 to December 2018, the clinical pregnancy rate and implantation rate were 30.8% and 21.61%, respectively. Although the risks of Oocyte cryopreservation were predicted and discussed, clinical criteria showed no difference between fresh and vitrification oocytes, which was due to the use of cryoprotectant (CPA) during oocyte freezing. Several factors have an impact on the pace of cooling, including the composition of the cell membrane, the ratio of the cell's surface area to

volume, the cooling temperature, and the conductivity of the cell membrane. The pace of cooling affects the amount of water lost during dehydration, and for human oocytes and embryos, a sufficient cooling rate allows for reasonably extensive membrane water exchange before all materials within and outside the cell solidify (Ahn *et al*., 2002). When CPA is introduced to oocytes in a freezing environment, it induces an osmotic gradient between the exterior and interior of the cell, causing water to relocate from the inside to the outside. Because of the osmotic pressure, CPA will replace water inside the cell. Since the rate at which water leaves the cell during freezing and returns to the cell during thawing is always faster than that of CPA substances, there is always a macromolecular CPA in the freezing medium to help CPA penetrate the membrane and maintain an osmotic pressure consistent with the osmotic rate of water (Stroh *et al*., 2002).

Conclusion

The quality of *in vitro* fertilization between vitrification and fresh oocytes was evaluated based on the morphology of each stage of embryo development, and the normal fertilization rate, embryo division rate, good - fair - moderate - poor embryo formation rate on 3rd day, blastocyst culture rate and good - fair - average blastocyst formation rate. The quality of oocyte cryopreservation will be evaluated based on clinical criteria with embryo transfer results, the number of embryos transferred and clinical data of pregnancy such as chemical pregnancy, clinical pregnancy, pregnancy progress, live births, and the number of miscarriages. In conclusion, this study showed no difference between the group of oocytes frozen by the vitrification method and fresh oocytes from the patients.

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