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Comparing the impact of different media on the development of human germinal vesicle oocytes recovered following intracytoplasmic sperm injection *in vitro*

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For patients enduring intracytoplasmic sperm injection (ICSI) cycles with controlled ovarian hyper stimulation or non-stimulated cycles, in vitro maturation (IVM) treatments have been used. Human tubal fluid, culture medium 199, and blastocyst media have all been subjected to IVM among other media. However, employing these media has a number of disadvantages, including a shorter shelf life and a greater cost for aided IVF clinics than regular culture media. IVM entails putting collected immature oocytes in a specific culture medium for maturation over a period of 1-2 days. The purpose of this study was to see how successful various types of IVM media were at increasing the number of mature oocytes available for fertilisation in ICSI cycles by increasing the number of germinal vesicles. The researchers used single-step culture media, homemade medium, and Sage IVM medium. This was done by watching oocyte maturation, fertilisation, and embryo development all the way to the blastocyst stage. Oocyte maturation was determined by looking at beneath an inverted microscope, the first polar body after 36 hours in culture. In order to inseminate ripe oocytes, intracytoplasmic sperm injection was performed (ICSI).The three groups' Following that, the rates of fertilization and embryo development were compared. In terms of statistical significance; there was a statistically significant difference between the three groups. Absence days, total motility, and the presence of abnormal morphology. The difference in ET amount between the two groups was statistically significant. the three groups, with the home made group having the greatest, followed by the global group, and then the sage group. IVM oocytes/total GV oocytes, normal fertilized oocytes/total GV oocytes, normal fertilized oocytes/IVM oocytes, Abnormal fertilized oocytes/IVM oocytes, IVM oocytes/IVM oocytes, IVM oocytes/IVM oocytes, IVM oocytes/IVM oocytes, IVM oocytes/ IVF oocytes, IVF oocytes, IVF oocytes, IVF oocytes, IVF oocytes, IVF oocytes, IVF oocytes, IVF Embryo arrest/fertilized oocytes, Embryo arrest/fertilized oocytes, Embryo arrest/fertilized. Because there is little or no ovarian stimulation and almost no risk of OHSS, the IVM is less costly, easier, and safer than normal aided conception therapy. It is the best choice for oocyte maturation at the GV Stage, and global media is the best option for culture.

Keywords: infertility in women, men, and In vitro maturation, intracytoplasmic sperm injection, and germinal vesicle acolytes.

INTRODUCTION

Infertility is a significant public health concern in both developing and developed countries. About one-fifth of all couples struggle with infertility. The failure of a couple to conceive is known as infertility. Following a year of unprotected sexual activity (Esmaeilzadeh et al. 2012).

Around 30% of infertility cases are caused by female causes. Because the female reproductive system is more intricate and less accessible, diagnosing and treating women may be more difficult than diagnosing and treating males. Age is still the most important factor in female infertility, while reproductive diseases can also play a role. Disorders of the ovaries, uterus, fallopian tubes, and other associated organs might interfere with ovulation, fertilisation, and implantation. Some women are diagnosed with a reproductive abnormality in their teens or early twenties, while others are unaware of the issue until they try to conceive. During a female infertility examination, certain symptoms or a family history of infertility will prompt testing for a reproductive condition. Two of the most Endometriosis and polycystic ovarian syndrome are two common causes of female infertility. (Wallace and Kelsey, 2010).

When direct treatment of the underlying issues isn't possible, assisted reproductive technology (ART) refers to clinical and laboratory procedures that are used to help infertile couples get pregnant. In vitro fertilisation, intracytoplasmic sperm injection, donor egg IVF,

preimplantation genetic diagnosis, aided hatching, and blastocyst transfer are some of the ART treatments that doctors, embryologists, and other technicians employ to cause eggs to fertilise, develop, and implant in the uterus. (Bagis et al. 2010).

IVF was first created to treat tubal infertility caused by Fallopian tube obstruction or damage. However, soon after its successful launch, its promise for treating Infertility in men has become a contentious topic. In the treatment of severe male infertility, intracytoplasmic sperm injection (ICSI) has supplanted normal IVF. (Schieve, 2006).

The procedure of inserting a single sperm into the cytoplasm is known as intracytoplasmic sperm injection. sperm into the cytoplasm of an egg (ICSI). In natural conception, a large number of sperm surround the egg, and many stick to the outside, but only one sperm penetrates and fertilises each egg. To mimic nature, a large amount of sperm is introduced to the dish holding the egg in classical IVF. ICSI is a technique that allows scientists to choose sperm based on their activity and appearance. A single sperm is then placed into each mature egg using specialized equipment that allows the egg and sperm to be managed precisely (NHS, 2017).

IVM is the process of obtaining developing immature oocytes from non-stimulated or weakly primed follicles Because there is little or no ovarian stimulation and almost no risk of ovarian hyper stimulation syndrome (OHSS), the IVM is less costly, easier, and safer than normal aided conception therapy (Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, 2013).

The medium utilised to assist IVM of the oocytes determines the success of IVM. MediCult IVM media as well as Sage IVM media frequently They've been utilised since they became commercially accessible, and they've shown to have high rates of fertilisation, implantation, and pregnancy. In vitro fertilisation the media, nevertheless, more expensive than regular media and culture (IVF). For IVM of immature mouse oocytes 199, Blastocyst medium performed as well as well as tissue culture medium and Sage IVM media (Kim et al. 2011).

Fesahat et al. (2017) evaluated the The meiotic development of metaphase I (MI) oocytes to full maturity was studied using four distinct culture mediums. at varied timeframes, including expert Media from IVM/IVF and DIY culture media. They came to the conclusion the IVM's quality medium influenced clinical IVM findings. In IVM/ICSI cycles, blastocyst medium might also be utilised as a substitute for IVM medium.

The purpose of this study is to see how well different types of IVM media work on germinal vesicles during ICSI cycles to enhance the number of mature oocytes available for fertilization. This was done by watching oocyte maturation, fertilisation, and embryo development all the way to the blastocyst stage.

MATERIALS AND METHODS

The oocytes of 150 infertile women were retrieved during the germinal vesicles stage and Three groups were formed:

The first group used a single-step culture medium that for maturized, fertilised, and cleaved oocytes (life global media.

The second group had their oocytes developed in Homemade IVM Media before being fertilised and cleaved in a global culture.

On the third group, oocytes were cultured in commercial medium (SAGE IVM Media) for maturation, fertilisation, and cleavage.

Inclusion criteria:

1. 35-year-old female
2. Did not have any medical problems, chronic illnesses conditions, or obstetric care concerns that may be jeopardised by extended an aesthetic or surgery.
3. Patients who have experienced IVF failures in the past.

Exclusion criteria:

1. Couples with a high male factor and testicular sperm samples.
2. Patient who has had a poor reaction and has had less than 5 oocytes harvested.
3. Women in their forties and fifties.

Materials:

Media:

1. Single-step culture media (life global): A bicarbonate-buffered medium containing glucose, lactate, pyruvate, and all 20 amino acids is optimal for supporting the growth and development of human embryos in vitro.
2. Homemade media: It's made up of 40% human follicular fluid and 75IU follicle stimulating hormone and 75IU luteinizing hormone mixed in with global media.

SAGE I*VM* Media.

1. HEPES Buffer Solution: HEPES Buffer Solution is a common buffer in cell culture media.
2. GM501 Hyaluronidase: This is a ready-to-use solution that aids in the mechanical removal of cumulus cells.
3. HSA-7 percent Poly-vinylpyrrolidone (PVP) Solution HSA-7 from FUJIFILM Irvine Scientific percent FUJIFILM Irvine Scientific is a division of FUJIFILM Corporation. Polyvinylpyrrolidone (PVP) Solution
4. FSH and LH ampoules, each FSH and LH levels are 75 IU each.

Methods:

Semen collection and preparation:

Sperm samples were taken after 3-5 days of sexual abstinence. collected via masturbation.WHO-

recommended density gradient method for sperm sample preparation (Nidcon, Sweden) (2010).

Oocytes retrieval and in vitro maturation:

1**.** Using a Wallace aspiration needle, eggs were extracted from 300 infertile women aged 35 during induced intracytoplasmic sperm injection.

2. Using a stereomicroscope and pipette, eggs were extracted from follicular fluid and placed in HEPES buffer medium.

3. GVs were chosen by removing a piece of the corona radiate and using Hyaluronidase to enclose the oocytes.

4. Using a sterile pipette, Gvs were put into three sets of equilibrated IVM Media and incubated in a double gas incubator.

The first category is referred to as GVs, which stands for "culture in life as a global media."

The second category is GVs Culture in a Homemade Medium.

The final group is GVs Culture in SAGE media.

The maturity was measured after 24 and 48 hours.

Intracytoplasmic sperm injection (ICSI):

Mature oocytes were moved to falcon (60x15mm) culture dishes with global media and inspected for injected oocytes by normal sperm using an inverted microscope and a micromanipulator.

Oocytes Culture and maturation:

The injected oocytes were grown in three distinct medium types: The first group cultivated the injected oocytes in life global media.

In the second group, the injected oocytes were grown in life global media.

In the third group, the injected oocytes were grown in SAGE IVM medium.

Fertilization (2 pronuclei) up till the blastocyst stage of embryo development was assessed 18 hours after ICSI.

Figure1: Fertilized oocyte with the 2 pro-nuclei

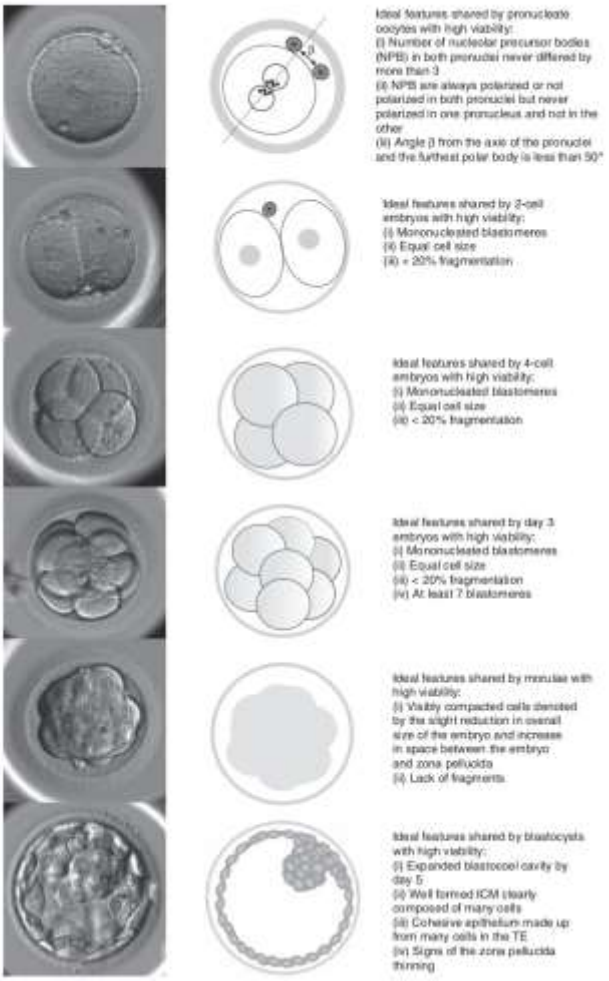

Figure 2: The embryo scoring (Gardner and Balaban, 2016)

RESULTS

Table 1 show the baseline and cycle parameters of the male spouse. On average, absenteeism is high. Days among male partners in global media were 3.61.26 days, 3.721.06 days in homemade media, and 3.501.38 days in sage media, with no statistically significant difference in mean abst days amongst the medias studied. In terms of sperm concentration, mean total motility was 22.26.60, 23.55.24, and 21.27.35 percent, mean progressive motility was 8.935.86, 8.735.83, and 9.095.89 percent, and mean aberrant morphology was 10.23.89, 9.154.60, and 11.003.00 percent in global, home produced, and sage media.

Table 2 shows the baseline features of the female spouse. The average age in global media was 28.74.26 years, 28.54.41 years in home generated media, and 28.84.14 years in sage media, with no statistically significant difference in mean age amongst the medias studied. Finally, in global, home created, and sage media, the mean number of harvested oocytes was 14.06.72,

14.67.33, and 13.66.08, respectively, and the mean number of injected MII was 8.724.12, 9.084.35, and 8.433.91, with There was no statistically significant difference across the medias that were evaluated.

Tables 3 and 4 show the pace of embryo growth in various IVM mediums as well as the maturation of oocytes at the GV stage after 24 to 48 hours following hand fertilisation. The percent of maturation in global media was 53.93 percent at 24 hours, 60.21 percent in home-made media, and 51.11 percent in Sage media; at 48 hours, it was 20.22 percent in global media, 22.58 percent in home-made media, and 17.77 percent in Sage media, with no statistical significance difference between the studied medias at 24 hours or 48 hours.

Table 1: Male partner baseline and cycle characteristics:

Variable	GLobal	Home made	Sage	Test	P value
Abst. days	3.60±1.26	3.72±1.06	3.50±1.38	1.16^	0.41 NS
Sperm concentration (10^6/ml)	24.1±19.9	23.5±18.6	24.6±20.8	**1.00^**	0.55 NS
Total motility (%)	22.2±6.60	23.5±5.24	21.2±7.35	0.48^	0.62 sNS
Progressive motility (%)	8.93±5.86	8.73±5.83	9.09±5.89	0.99^	0.49 sNS
Morphology (abnormal %)	10.2±3.89	9.15±4.60	11.00±3.00	0.86^	0.43 sNS

Data presented as mean and SD or percent
Test: ^:F (ANOVA) $:chi square test, $:chi square test
NS stands for non-significant (P>0.05).

Table 2: Baseline and cycle Characteristics of the female parteners of the researched medias

Variable	GLobal	Home made	Sage	Test	P value
Age	28.7±4.26	28.5±4.41	28.8±4.14	0.98^	0.37
No. of Oocytes collected	14.0±6.72	14.6±7.43	13.6±6.08	1.02^	0.103
No of MII injected	8.72±4.12	9.08±4.35	8.43±3.91	1.04^	0.085

Data presented as mean and sd or percent
Test: ^:F (ANOVA) test, $:chi square analysis
NS stands for non-significant (P>0.05).

Table 3: Oocyte maturation at the GV stage after 24 hours, as well as fertilisation and embryo formation rates in various IVM media:

Media	After 24 hr			
	Maturation	**Fertilization**	**Embryo Division**	**Embryo Blastula ion**
Global	48/89 (53.93%)	27/48 (56.25%)	25/27 (92.59%)	13/25 (52%)
Home made	56/93 (60.21%)	34/56 (60.71%)	33/34 (97.05%)	19/33 (57.5%)
Sage	46/90 (51.11%)	21/46 (45.65%)	18/21 (85.71%)	9/18 (50%)
P Value	NS = 0.44	NS= 0.30	NS= 0.29	NS =0.85

Data presented as number and percent
Test: chi square analysis
NS stands for non-significant (P>0.05).

Table 4: Maturation of oocytes at GV stage after 48 and their fertilization and embryo formation rate in different IVM media

Media	After 48 hr			
	Maturation	**Fertilization**	**Embryo Division**	**Embryo Blastulation**
Global	18/89 (20.22%)	9/18 (50%)	8/9 (88.88%)	3/8 (37.5%)
Home made	21/93 (22.58%)	12/21 (57.14%)	11/12 (91.66%)	5/11 (45.4)
Sage	16/90 (17.77%)	7/16 (43.75%)	6/7 (85.71%)	2/6 (33.3%)
P Value	NS= 0.72	NS= 0.71	NS= 0.92	NS= 0.87

Data presented as number and percent
Test: chi square analysis
NS stands for non-significant (P>0.05).

Table 5: After cultivation in various IVM medium, The development of GV oocytes was studied in terms of nuclear maturation, fertilisation, embryo formation, and developmental status.

Variable	GLobal	Home made	Sage	The p-value
IVM oocytes as a percentage of total GV oocytes maturation	66/89 (74.15%)	77/93 (82.79%)	62/90 (68.88%)	0.16 NS[1] 0.43 NS[2] **0.03*[3]**
Fertilized oocytes as a percentage of total GV oocytes	36/89 (40.44%)	46/93 (49.46%)	28/90 (31.11%)	0.22 NS[1] 0.19 NS[2] **0.01*[3]**
Normal fertilized oocytes/IVM oocytes	36/66 (54.54%)	46/77 (59.74%)	28/62 (45.16%)	0.53 NS[1] 0.29 NS[2] 0.09 NS[3]
Abnormal fertilized oocytes/IVM oocytes	5/66 (7.57%)	5/77 (6.49%)	6/62 (9.67%)	0.80 NS[1] 0.67 NS[2] 0.49 NS[3]
Formed embryos/total GV oocytes	33/89 (37.07%)	44/93 (47.31%)	24/90 (26.66%)	0.16 NS[1] 0.14 NS[2] **0.004*[3]**
Formed embryos/IVM oocytes	33/66 (50%)	44/77 (57.14%)	24/62 (38.70%)	0.39 NS[1] 0.19 NS[2] **0.03*[3]**
Formed embryos/fertilized oocytes	33/41 (80.48%)	44/51 (86.27%)	24/34 (70.58%)	0.46 NS[1] 0.32 NS[2] 0.08 NS[3]
Embryo arrest/fertilized oocytes	5/41 (12.19%)	4/51 (7.84%)	5/34 (14.70%)	0.48 NS[1] 0.75 NS[2] 0.32 NS[3]
High-quality embryos	12/33 (36.36%)	17/44 (38.63%)	10/24 (41.66%)	0.84 NS[1] 0.68 NS[2] 0.81 NS[3]

Data presented as number and percent
Test: chi square test
NS: P>0.05 indicates that the difference is not significant.
*:Statistically significant (P0.05) ** Statistically significant (P0.001)

Fertilization of mature oocytes was 56.25 percent in global media, 60.21 percent in homemade media, and 45.65 percent in Sage media after 24 hours, and 50 percent in global media, 57.14 percent in homemade media, and 43.75 percent in Sage media after 48 hours, with no statistical significance difference between medias. In terms of the rate at which embryos are produced.

92.59 percent, 97.05 percent, and 85.71 percent in global, homemade, and sage media, respectively; 88.88 percent, 91.67 percent, and 85.71 percent in global, homemade, and sage media, respectively; and 47.5 percent, 45.4 percent, and 33.3 percent in global, homemade, and sage media, respectively, with no statistical significance difference; and 47.5 percent, 45.4 percent, and 33.3 percent in global, homemade.

After incubation in various IVM medium, nuclear maturation, fertilisation, embryo formation, and developmental status of GV oocytes are shown in Table 5. In global media, the percentage of IVM oocytes/total GV oocytes was 74.15 percent, 82.79 percent in home generated media, and 68.88 percent in sage media, with a statistically significant increase in % in home manufactured media compared to sage media. The proportion of normal fertilised oocytes/total GV oocytes in global media was 40.44 percent, 49.46 percent in home manufactured media, and 31.11 percent in Sage media, with homemade media having a statistically significant gain over Sage media. With no statistically significant difference across the media types, the percentage of normal fertilised oocytes/IVM oocytes in global media was 54.54 percent, 59.74 percent in homemade media, and 45.16 percent in Sage media. The percent of formed embryos/total GV oocytes was 37.07 percent, 47.31 percent, and 26.66 percent in global, homemade, and sage media, respectively, and the percent of formed embryos/fertilized oocytes was 50 percent, 75.14 percent, and 38.70 percent in global, homemade, and sage media, with a statistically significant increase in percent of both in homemade media compared to sage media. Finally, the percent of formed embryos/fertilized oocytes was 80.48 percent, 86.27 percent, and 70.58 percent in global, homemade, and sage media, respectively, while the percent of embryo arrest/fertilized oocytes was 12.19 percent, 7.84 percent, and 14.70 percent, and the percent of high-quality embryos was 36.36 percent, 38.63 percent, and 41.66 percent in global, homemade, and sage media

DISCUSSION

IVF with maturation in the laboratory (IVM) a number of oocytes offers being able to produce a big one number of embryos in a secure and cost- effective manner. It does not need superovulation or synchronisation, and it poses

little danger of ovarian hyperstimulation syndrome. Even from enhanced IVF cycles, IVM requires providing embryo development in a controlled environment for transgenics, cloning, and stem cell research (Practice Committees of the American Society for Reproductive Medicine, 2013).

Following stimulation of the ovaries, around During the germinal vesicle (GV) and GV breakdown (maturity [MI]) phases, 15% of the recovered oocytes are immature. IVM might make it more likely for patients to use as many of their oocytes as possible in IVF treatments. Because low egg rates of maturation and embryo development during IVM are regarded as significant. issues, selecting the right culture medium is crucial to IVM success (Combelles and Chateau, 2012).

In several clinical and scientific studies, Human tubal fluid (HTF), culture medium 199, and blastocyst medium are all examples of human tubal fluid. Have all been used as IVM media. MediCult and Sage IVM media there are two types of IVM media that are commercially accessible. that are regularly used and have good fertilisation, implantation, and pregnancy rates (Kim et al., 2011).

However, there are several disadvantages to utilising these specially prepared commercial media, such as the need to order ahead of time, the short the fact that they have a longer shelf life, and the fact that they are more expensive than regular For assisted IVF, cultural media institutes in which IVM procedures don't take place regularly. Blastocyst medium was shown to be appropriate for human immature oocyte IVM in un-stimulated IVF cycles when compared to Sage IVM media (Pongsuthirak et al. 2015).

As a result, the typical assisted conception facility has a number of obstacles, as IVM services are not often given. Immature mouse oocytes were used for IVM. 199, Blastocyst medium performed as well as well as tissue culture medium and Sage IVM media (Kim et al., 2011).

This study evaluates the level of effectiveness several types of IVM media on germinal vesicles in ICSI cycles to induce intracytoplasmic sperm injection maximise the amount of oocytes that have reached maturity available for prospective fertilisation. The researchers used a one-step culture media (life global medium), a home-made medium, and a commercial medium (Sage IVM medium).

This was done by watching oocyte maturation, fertilisation, and embryo development all the way to the blastocyst stage. Oocyte maturation was determined by looking at beneath an inverted microscope, the first polar body after 36 hours in culture. Using a denuding pipette and 80 IU/mL hyaluronidase for 30 seconds in flushing medium, surrounding cumulus cells were removed prior to maturity testing. In order to inseminate ripe oocytes, intracytoplasmic sperm injection was performed (ICSI). The three groups' rates of fertilisation and embryo development were then compared.

When the baseline and cycle data of male partners in each group were examined, the mean of absenteeism days among male partners in global media was 3.6±1.26 days, 3.72±1.06 days in home created media, and 3.50±1.38 days in sage media, with no statistical significant difference between the medias analysed.

In the global, homemade, and sage media, mean sperm concentrations were 24.1±19.9, 23.5±18.6, and 24.6±20.8 (106/ml), mean total motility was 22.2±6.60, 23.5±5.24, and 21.2±7.35%, mean progressive motility was 8.93±5.86, 8.73±5.83, and 9.09±5.89 percent, and mean abnormal morphology was 10.2±3.89, 9.15±4.60, and 11.00±3.00 percent.

When the baseline and cycle data of female partners within each group were examined, the mean age was 28.74.26 years in global media, 28.54.41 years in home manufactured media, and 28.84.14 years in sage media, with no statistically significant difference in mean age across the three medias. Finally, in global, home made, and sage media, the average number of harvested oocytes was 14.06.72, 14.67.33, and 13.66.08, respectively, and the average number of injected MII was 8.724.12, 9.084.35, and 8.433.91 in both, with no statistically significant difference across the tested medias.

Patient characteristics, according to Pongsuthirak et al. (2015), were age Body mass index 28.7 + 4.7 kg/m2 (range 19.0-47.4 kg/m2), gravida 1.6+0.6 (range 1-4), parity 0.5 + 0.6 (range 0-3), gestational age 38.3 + 0.9 weeks (range 37-40 weeks), and gestational age 38.3 + 0.9 weeks (range 37-40 weeks). Blastocyst medium and Sage IVM media (Group I) (group II) were used to separate the oocytes of each individual (group II). Despite the fact that Only 1032 of 1178 immature oocytes satisfied the criteria. the study's eligibility criteria and were included (In group I, there were 512 oocytes, and in group II, there were 520 oocytes.)

A variety alterations in nuclear and cytoplasmic structure currently unknown impede oocyte development. Because sustaining The term "meiotic arrest" refers to a condition in which the a process It has to do with cooperation of the whole follicle, nuclear maturation takes place naturally when Oocytes that are ready to be implanted are withdrawn from their follicular surroundings. (Meintjes, 2012).

Many cytoplasmic organelles change their shape and distribution, and messenger RNA transcripts reactivate to store proteins and transcripts necessary for early embryonic development until the genome is activated (Hennet and Cambell, 2012).

At 24 hours, the percent of maturation in global media was 53.93 percent, homemade media was 60.21 percent, and Sage media was 51.11 percent, while at 48 hours, it was 20.22 percent in global media, 22.58 percent in homemade media, and 17.77 percent in Sage media, with no statistically significant difference between the studied medias.

After 24 hours, the percent of matured oocytes fertilised was 56.25 percent in global media, 60.21 percent in homemade media, and 45.65 percent in Sage media; after 48 hours, it was 50 percent in global media, 57.14

percent in homemade media, and 43.75 percent in Sage media, with no statistical significance difference between medias in mature oocyte fertilisation after 24 hours or 48 hours. When it comes to the rate at which embryos develop.

At 24 hours, 92.59 percent, 97.05 percent, and 85.71 percent in global, homemade, and sage media, respectively; at 48 hours, 88.88 percent, 91.67 percent, and 85.71 percent in global, homemade, and sage media, respectively; and at 48 hours, 88.88 percent, 91.67 percent, and 85.71 percent in global, homemade, and sage media, respectively; and percent of embryo blastulation at 24 hours,

Walls et al. (2012) found a maturation rate of 44 percent to 67 percent and a fertilisation rate of 40 percent to 77 percent using various IVM media following a 28-36-hour in vitro culture. Son et al. (2005) obtained IVM oocytes during the follicular phase (41.2 percent -47.4 percent).

In terms of At 36 hours, the rate of maturation has reached a peak., ICSI fertilisation rate, rate of cleavage oocytes that have been fertilised, and Blastocyst creation rate, Pongsuthirak et al. (2015) There was no statistically significant difference between the two groups.

The % Maturation, IVM oocytes/total GV oocytes in global media was 74.15 percent, 82.79 percent in home produced media, and 68.88 percent in sage media in our study, with a statistically significant increase in percent in home manufactured media over sage media. The proportion of normal fertilised oocytes/total GV oocytes in global media was 40.44 percent, 49.46 percent in home manufactured media, and 31.11 percent in Sage media, with homemade media having a statistically significant gain over Sage media. With no statistically significant difference across the media types, the percentage of normal fertilised oocytes/IVM oocytes in global media was 54.54 percent, 59.74 percent in homemade media, and 45.16 percent in Sage media.

The global, homemade, and sage media had 7.57 percent, 6.49 percent, and 9.76 percent, respectively, of aberrant fertilised oocytes/IVM oocytes, with no statistical significant difference between them. The percent of formed embryos/total GV oocytes was 37.07 percent, 47.31 percent, and 26.66 percent in global, homemade, and sage media, respectively, and the percent of formed embryos/fertilized oocytes was 50 percent, 75.14 percent, and 38.70 percent in global, homemade, and sage media, with a statistically significant increase in percent of both in homemade media compared to sage media. Finally, the percent of formed embryos/fertilized oocytes was 80.48 percent, 86.27 percent, and 70.58 percent in global, homemade, and sage media, respectively, while the percent of embryo arrest/fertilized oocytes was 12.19 percent, 7.84 percent, and 14.70 percent, and the percent of high-quality embryos was 36.36 percent, 38.63 percent, and 41.66 percent, with There is no statistically significant difference between the two media.

In IVM cycles, there is no consensus on the ideal timing for oocyte maturation, and numerous approaches have been outlined. Cleavage and blastocyst development rates were significantly lower 72.2 percent of mature oocytes after 48 hours of IVM, 19.0 percent, compared to those who were grown 24 hours after IVM (72.2 percent, 19.0 percent), according to Son et al. (2005). (91.5 percent , 50.4 percent). They stated that in an IVM, oocytes that enter metaphase II sooner regimen had increased embryonic development developmental ability. According to Farsi et al. (2013), oocytes that had not yet matured developed between 24 and 30 hours in stimulated ICSI cycles before being After the polar body, the polar body was injected for continued cultivation. was extruded.

Fesahat et al. (2017) investigated how different media influenced immature oocytes' IVM outcomes GV stands for germinal vesicle. During stimulated intracytoplasmic sperm injection (ICSI) cycles, 400 immature oocytes with normal morphology at the GV stage were collected from 320 infertile women aged 314.63 years. They were developed in groups of 100 for 24 to 48 hours at 37°C in homemade IVM medium (I), cleavage medium (II), blastocyst medium (III), and Sage IVM media (IV).

The maturity rates of the 400 GV oocytes were measured.varied significantly between groups I and IV (55 percent, 53 percent, 78 percent, and 68 percent, respectively); a higher proportion (78 percent) Group III had more of it than Group IV. but the difference It didn't matter. Furthermore, oocytes from groups I and II with varying incubation times periods matured at similar rates (38 percent and. 43% in the 24-hour group and 17% in the 48-hour group (vs. 10% in the 48-hour group). Although blastocyst and Sage IVM media generated greater rates, total fertilisation Groups I and II had the highest rates. similar (52.7 percent and 56.6 percent, respectively). Overall fertilisation IVM oocyte production rates and the pace at which incorrectly fertilised oocytes in IVM oocytes (with one or more pronuclei) did not differ significantly ($p=0.1$ and $p=0.9$, respectively) in both media groups (Fesahat et al., 2017).

There were no noteworthy events. variations in the womb growth or the number of arrests between the media groups among the fertilised oocytes. On the other hand, the group given Sage IVM medium had the highest rate of embryo development. Despite There are no substantial changes in fertilisation and embryo development. formation The majority of GV maturation was finished within 24 hours 38 percent of the time in both groups., 43 percent, 70 percent, Rather than 48 hours (17 percent, 10%, 8%, and 15% in groups I–IV, respectively), participants were given 48 hours (17 percent, 10%, 8%, and 15% in groups I–IV, respectively). Furthermore, compared to the other groups, group IV showed a higher quality rate embryo development (as well as grades A and B). Furthermore, embryos produced by oocytes cultivated Oocytes grown in blastocyst or Sage produced poorer

quality results in homemade and cleavage medium. medium, with the majority falling into classifications C and D. (Fesahat and colleagues, 2017).

Only a few researches have looked at the effects of various IVM medium on immature oocytes derived from stimulated or non-stimulated cycles. In two randomised controlled experiments employing immature human oocytes retrieved following caesarean deliveries, Pongsuthirak et al. (2015) and Pongsuthirak and Vutyavanich (2015) compared MediCult IVM media (Origio) with Sage IVM media (Cooper Surgical), as well as blastocyst and Sage media. There were no differences in oocyte maturation (65% vs. 69%) or fertilisation (65.2 vs. 69%) between the two medium groups ($p>0.05$). They came up with the idea that immature oocytes collected during pregnancies may develop. The choice of Medicult or Sage IVM medium is dependent on availability, price, and convenience of use, despite the fact that their efficacy is identical. This technique, which may be used to make mature products, This method is a viable alternative to oocyte donation for producing mature oocytes for stem cell research.

Gardner et al. (1994) used a variety of Maturation rates of 44 to 67 were reported by IVM media. Percent and fertilisation rates of 40 to 77 percent in vitro cultivation for 28 to 36 hours They discovered that 45.9% to 46.6 percent of blastocysts produced were of good quality. After testing for two pronuclei, the endpoint was determined exclusively by embryo development during the cleavage stage, and the percentage of high-quality embryos (day 3).

In our study, 75 IU luteinizing hormone and 75 IU follicle-stimulating hormone were given to every IVM media, potentially increasing the number of follicles. production of meiosis-activating chemicals and hence boost oocyte maturation rate. The cleaved and prepared media contain Proteins found in plasma Important amino acids and monobasic anhydrous potassium phosphate are missing from these media., which may affect the oocyte maturation process as compared to blastocyst and Sage media.

Farsi et al. (2013) assessed the formulae of various media and discovered that taurine and calcium, which are unique to blastocyst medium, were likely useful for IVM media in a review study. Because they are key antioxidants found in abundance in gametes and the embryonic environment, taurine and hypotaurine were assumed to be helpful. Calcium lactate in blastocyst culture conditions may also boost mitochondrial function by assisting in the generation of sufficient adenosine triphosphate. When comparing commercial IVM media to normal growth medium for enhancing the maturation of oocytes produced from stimulated cycles, Moschini et al. (2011) discovered that commercial IVM media offer no substantial advantages.

De Araujo et al. (2009) for different types of patients, other basic or modified media were employed TCM-199 culture medium was found to be superior to HTF-supplemented medium for oocyte maturation, fertilisation, and subsequent embryo development in patients with polycystic ovary syndrome (PCOS); they reported findings similar to those of the current study in terms of maturation rate (56.9% vs. 55.5%), fertilisation rate (39.4% vs. 53%), and embryo quality (56.9% vs. 53%). (56.9% and. 53%). (56.9 percent vs. 53 percent). (41.7 percent and. 28.5 percent), respectively. HTF medium fails to mature oocytes retrieved from PCOS patients during non-stimulated cycles, according to the researchers. TCM 199 surpassed HTF medium in the IVM of oocytes obtained from PCOS patients in terms of maturation rate (82 percent vs. 56.9%), fertilisation rate (70 percent vs. 39.4 percent), and embryo quality. (70 percent vs. 39.4 percent). (81.3 percent vs. 41.7 percent), respectively.

When compared to Sage and blastocyst media, Kim et al. (2011) concluded TCM-199 was used as a culture medium inferior. These findings appear to be connected to IVM formulations that are non-specific and basic, and that such formulations are unable to fulfil all of the immature oocytes' cytoplasmic and nuclear maturation in vitro demands. In our study, the early stages of inadequate oocyte maturation may be a key factor due to poor IVM results in the future processes of fertilisation as well as embryo development in the third and fourth groups The evidence backs this up. fact found the rates of embryo development in groups I and II were rather high in oocytes that had successfully completed the cytoplasmic and nucleic maturation processes. fertilised. Meintjes (2012) also discovered suggesting plasma protein supplementation can provide further benefits components that help regulate oocyte maturation.

Because each ICSI cycle produced a variable number of immature oocytes, the groups were assigned based on the number of oocytes each group, each containing one normal oocyte Furthermore, we were unable to assess the clinical effects of this experiment since The immature oocytes retrieved from stimulated cycles are not the same as those recovered from normal cycles. Commonly are utilised in patients and consequently destroyed. We, on the other hand, endeavoured to limit mistakes made by addressing numerous features of research design, such as determining sample size for at least a 50% maturation rate of immature oocytes making use of any medium, establishing oocyte selection ICSI should be performed by a single expert, and inclusion criteria should be followed. Furthermore, definitive studies with relation to the foundation of human IVM Currently, imprinting is in use.unavailable.The IVM medium's high quality has an influence on clinical IVM findings, according to Fesahat et al. (2017). Additionally, blastocyst medium might be used in IVM as an alternate IVM medium /ICSI cycles.

CONCLUSION

In our research, we discovered that harvesting oocytes after a caesarean birth is both safe and effective.

It's a viable alternative to traditional oocyte donation, as well as a possibility for people who have become pregnant as a consequence of ART therapy in the past and wish to continue However; there are no more cryopreserved oocytes/embryos available for therapy. This approach might be utilised to make mature oocytes for stem cell research and fertility therapies.

We arrived at the following findings after adding various IVM media to GVs:

This was one of the first studies to compare one step culture media (life global medium), home-made medium, and commercial ICSI (induced intracytoplasmic sperm injection) cycles require a medium. (Sage IVM medium).

Why The IVM is less costly, easier to use, and more secure than other options standard conception with the help of technology therapy since There is little or very little ovarian stimulation, and the risk of OHSS is almost non-existent.

The IVM media's quality has an impact on clinical IVM results.

Home-made IVM Media is the best option for oocyte

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

Amoura M. Abou El-Naga: Suggested the work protocol, Analyzed and interpreted the data, Revised the manuscript. Ahmed M. Badawy: Conceived and designed the experiments. Salma M.T. Elmetwally: Performed the laboratory experiments, Wrote the paper.

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