Original Article

To cite: Raghunandan K, Tibrewal A, Rawat B, Dey P. Effect of Embryo Glue as a Transfer Medium in the Outcome of Implantation Rate and Live Birth Rate in Freeze-Thaw Embryo Transfer Cycles. Pan Asian J Obs Gyn 2021;4(1):14-21.

Received on:
17-11-2020

Accepted on:
04-02-2021

Effect of Embryo Glue as a Transfer Medium in the Outcome of Implantation Rate and Live Birth Rate in Freeze-Thaw Embryo Transfer Cycles

[1]Raghunandan K, [2]Anuradha Tibrewal, [3]Beena Rawat, [4]Paulami Dey

[1]Senior Clinical Embryologist, [2,4]Consultant, [3]Embryologist

[1]Department of Embryology, Genome-The Fertility Centre, Raipur, Chhattisgarh, India
[2,4]Department of Reproductive Medicine, Genome-The Fertility Centre, Raipur, Chhattisgarh, India
[3]Department of Embryology, Ferticity Fertility Clinics, Delhi, India

ABSTRACT

Background: Embryo Glue is a Hyaluronan enriched transfer medium, which aids in the implantation rate by enhancing uterine receptivity. Advance maternal age is an important factor that negatively influences the implantation rate in assisted reproductive technology because the rate of implantation decreases with the patient's age.

Aim: The aim of the study is to evaluate whether the transfer of embryos using embryo glue improves the implantation rates outcome and live birth rate in freeze-Thaw cycles.

Materials and Methods: It was a retrospective study. A total of 391 patients who had undergone frozen-thawed embryo transfers were recruited. Patients who had embryo transfer with hyaluronan-enriched transfer medium (HETM) were grouped as Embryo glue (n = 207, Test group) and patients who had embryo transfer with standard ET medium grouped as without Embryo glue (n = 184, control group) were divided. Patients were further divided into two groups (as per age) as young (Age = ≤34 years) and AMA (Age = ≥35 years). The pregnancy was confirmed by measuring beta-human chorionic gonadotropin (beta-hCG) level after 14 days of embryo transfer. The chi-square test was preformed to analyze outcomes.

Results: Overall implantation rate (IR), multiple pregnancy rate (MPR), and live birth rate (LBR) significantly increased with the use of embryo glue as an embryo transfer medium [IR: 19.5% vs. 24.5% (P = 0.01), MPRs: 19.0% vs. 28.1% (P = 0.028), LBRs: 65.4% vs. 80.0% (P = 0.005), for control groups and Embryo Glue, respectively]. The beneficial effect was significant in women who were >35 years of age [IRs: 11.2% vs. 16.8% (P = 0.019)].

Conclusion(s): The enrichment of transfer medium with hyaluronan increases IRs, for frozen-thawed embryo transfer. The beneficial effect was most evident in women who were >35 years of age, the use of embryo glue in routine practice is safe and beneficial.

Keywords: Assisted reproductive technology, embryo glue, advance maternal age, frozen embryo transfer, implantation rate.

INTRODUCTION

Assisted reproductive technique (ART) is the mainspring of infertility treatment nowadays and fulfilling the wish of parenthood of many couples. *In vitro* fertilization (IVF) and embryo transfer (ET) has become favored and the pregnancy rates are enhanced by the blooming of the technology. The success and failure in ART have largely been ascribed to variables, i.e., patient's age, weight, endometrial receptivity, embryo quality, culture system, personnel skills, and also the techniques and medium used for embryo transfer (ET). All the above-mentioned variables can lead to a successful pregnancy if they are properly treated before/or during the IVF cycle or else they can also result in unsuccessful implantation.

Address for Correspondence
Raghunandan K
Senior Clinical Embryologist
Department of Embryology
Genome-The Fertility Centre
Raipur, Chhattisgarh, India
raghukempaiah92@gmail.com

There are many parameters that were considered a contributor to the implantation failure in a woman during IVF but one of the most common is a failure in developing interaction between the sticky matrix of the endometrium and the transferred embryo in the uterus. Also, the reproductive capacity in women declines dramatically with the advanced maternal age (≥35 years).[2] It is proven that spontaneous cumulative pregnancy rates begin to decrease at the of age 35–39 years and approach almost zero soon after 45 years. The advanced maternal age (AMA) corresponds with a decrease in both oocytes quantity and quality, and an increased rate of chromosomal aneuploidy in the embryos, which correlates with lower fertilization rates, poor embryo development, decreased implantation and pregnancy potential of women.[6] AMA can also hamper the makeover of the uterus environment which may lead to implantation failure as well.

The constituent of the female reproductive tract contains many micro- and macromolecules like albumin as the most abundant macromolecule which facilitate the process of implantation and helps in the fetal development as well. As like albumin, the hyaluronan also presents in a higher concentration which makes the matrix of uterus sticky and suitable for implantation by allowing the adhesion of the transferred embryo to the endometrium.[8]

Over a decade, there are many modifications emerge in the in vitro culture system to ensure the maximum clinical results. Generally, the media used for embryo culture and transfer are almost aqueous with supplemented proteins like human serum albumin (HSA) or whole serum, but these supplements are not that much viscous as fluid present in the female reproductive tract which provides a viscous sticky matrix for the embryo attachment.[1] To overcome the problem of sticky matrix embryo interaction, many protein supplements have been widely used in the ET medium.

Hyaluronan is a macromolecule with high relative molecular mass linear polysaccharide, comprised of repetitive units of *N*-acetyl *D*-glucosamine and *D*-glucuronic acid.[3] The Hyaluronan enriched culture medium is hypothesized to be best suited to embryo and blastocyst development in vitro. Hyaluronan interrelates in an autocrine and paracrine manner and also with certain cell surface receptors like CD 44 expressed on preimplantation embryo and in the endometrium. Hyaluronan is also known to be involved in physiological processes like embryological development, and adhesion. It also proliferates viscosity, which promotes easy handling, facilitating the embryo transfer process, preventing the deportation of embryos from the uterine cavity post transfer.[10]

The embryo glue has a higher concentration of hyaluronan and a lower amount of albumin which helps in the process of implantation by facilitating the adhesion of the embryo with the endometrium lining of the uterus. Hence, Embryo Glue is thought to be the ideal medium for embryo transfer. Accordingly, the clinical study was attempt to gauge the potency of the embryo glue as an embryo transfer medium.

The study is to evaluate the efficacy of the embryo glue as an embryo transfer medium in terms of implantation rate, overall pregnancy rate and live birth rate in frozen embryo transfer (FET) cycles.

MATERIALS AND METHODS

Study Design

The aim of this study is to evaluate the efficacy of the embryo glue as an embryo transfer medium in terms of implantation rate, overall pregnancy rate and live birth rate in frozen embryo transfer (FET) cycles.

SUBJECTS AND METHODS

Study Design

This is a retrospective controlled study conducted in a private IVF setup.

Inclusion Criteria

Patients included in this study from the year 2017-2020 who were selected for Intracytoplasmic sperm injection-Frozen embryo transfer (ICSI - FET) (Day 3).

Patients with hormonal and structural anomalies like poor ovarian reserve (POR), tubal factor, male factor, polycystic ovarian syndrome (PCOS) and unexplained infertility were included in this study. Informed consent has been taken from the recruited patients.

Exclusion Criteria

Patients with ICSI—Fresh embryo transfer (ET), IVF, male patients with azoospermia, or undergone any

surgical sperm retrieval (TESA, PESA, TESE, Micro TESA) were excluded from this study.

Methodology

The stimulation protocol was followed by down-regulation with GnRH antagonist (Cetrolix acetate, INTAS pharmaceuticals) depending on the patient's age, body mass index (BMI), and previous response (If any). A recombinant menopur (FERRING pharmaceuticals) was given to the patients for 10-12 days until the size of the follicles reaches 12-14 mm. The follicular development was continuously monitored by transvaginal sonography along with estradiol (E2) levels in the blood. Once the follicles reach the size of 18-22 mm, the human chorionic gonadotropin (hCG) was administered. Oocytes retrieval was performed 34-36 h of hCG trigger under general anesthesia with the help of TVS by suction.

Follicular fluid was screened under a stereo zoom microscope. The cumulus-oocyte complexes (COCs) is pre-washed in equilibrated (37°C) buffer media (G-MOPS, Vitrolife, Sweden), and oocytes shifted to pre-equilibrated (37°C, 6% CO_2 and 5% O_2) dish containing supplemented fertilization media (G-IVF, Vitrolife, Sweden). The culture dish was incubated for 2-3 hours. Meanwhile, a fresh semen sample from a male partner was obtained by masturbation and prepared by using the density gradient (DG) method. Cumulus oocytes cell removed two to three hours post ovum pickup (OPU) using the enzymatic method hyaluronidase (Sage IVF Inc., 80 U/ML). All matured metaphase II (MII) oocytes then separated and ICSI was performed on all MII oocytes by injecting morphologically normal sperm. All oocytes were incubated in a cleavage medium ("G-series" produced by vitrolife, Vitrolife, Sweden) in a petri dish at 37°C, 6% CO_2 and 5% O_2 post-ICSI.

Fertilization was observed 16-18 hours post-ICSI. The fertilization was confirmed by observing two distinct Pro-nuclei in the center of the oocytes and two Polar bodies in the peri-vitelline space. The embryos were further cultured in the same cleavage medium till day 3 as per the standard protocol.

On day 3, the embryo assessment was performed, and embryos were divided as Grade A or 1 and grade B or 2 as per the grading system (Istanbul consensus) (Fig. 1). All Grade A/1 and B/2 embryos were cryopreserved on day 3 with the vitrification method (Kitazato Bio-Pharm, Tokyo, Japan) in cryo locks.

Grade	*Rating*	*Description*
1.	Good	• <10% fragmentation
		• Stage-specific cell size
		• No multinucleation
2.	Fair	• 10-25% fragmentation
		• Stage-specific cell size for majority of cells
		• No evidence of multinucleation
3.	Poor	• Severe fragmentation (>25%)
		• Cell size not stage specific
		• Evidence of multinucleation

Source: The Istanbul consensus workshop on embryo assessment.

Fig. 1: The grading system of embryos (Instanbul Consensus)

Embryo Transfer

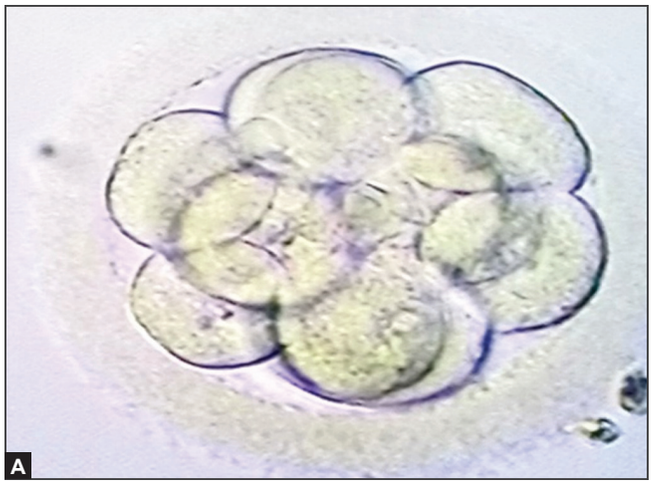
For the FET cycle, the endometrium was prepared by giving progesterone supplements and once the endometrium reaches the adequate tri-laminar stage (6 mm-12 mm) monitored by Doppler imaging, the FET was planned.

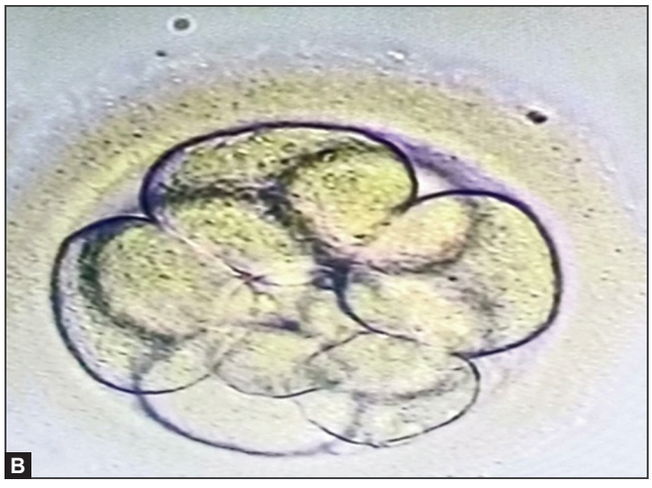
On the day of transfer, the embryos were thawed by using a kitazato thawing kit (Kitazato Bio-Pharm, Tokyo, Japan). Embryos were incubated for 2 hours post-thaw to check the survival. The control group (Standard ET medium) embryos were cultured and transferred in G2 plus (G-2, Vitrolife, Sweden) medium, and test group (Embryo Glue) embryos were cultured and transferred into hyaluronan-enriched transfer medium (500 uL) (Embryo Glue, Vitrolife, Sweden). Minimum two grades A/B day3 embryos (Figs. 2A and B) were transferred into the patient uterus with the help of cook embryo transfer catheter (Sydney IVF embryo transfer set, cook, Bloomingtom, IN, USA). The whole procedure was monitored by trans-abdominal ultrasound guidance.

Outcome Measures

Fourteen days after, the pregnancy was confirmed based on serum Beta hCG levels.

The pregnancy rate was calculated on the base of serum Beta hCG levels (>30 mIU/mL). The implantation of embryos was confirmed after 2 weeks of beta hCG positive results by observing gestational sacs in the uterine cavity with the help of transabdominal ultrasonography.





Figs. 2A and B: Image of embryos transferred. (A) Even size of blastomeres with zero degree of fragmentation. (B) A little uneven in blastomeres size.
Source: Genome-The Fertility Centre, Raipur

$$\text{The implantation rate} = \frac{\text{Number of gestational sacs seen}}{\text{Number of embryos transferred}} \times 100$$

Statistical Analysis

The statistical analysis was performed by using SPSS Statistics Software (SPSS Inc, Chicago, IL, USA). Variables are presented in percentages as well as numbers. The chi-square test was performed to analyze the outcome, the differences were considered significant at P <0.05.

RESULTS

In this study, the total number of 391 patients were recruited under inclusion criteria. A number of patients who have undergone FET with embryo glue as an embryo transferring medium grouped as Test (n = 207)

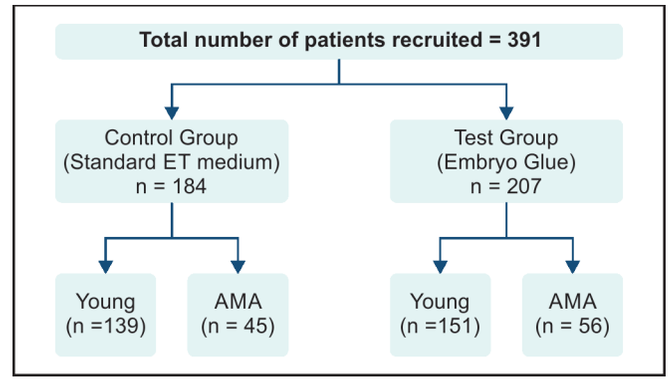


Fig. 3: Representing the number of patients (n) recruited in each group

and the rest of the patients who have undergone FET with standard embryo transfer media (G-2, Vitrolife, Sweden) grouped as Control (n = 184). Characteristics of patients were suballocated into two groups as young (Age = ≤34 years) and AMA (Age = ≥35 years) and comparison had made between the groups **(Fig. 3)**.

The overall implantation rate was observed significantly increased in the test group (embryo glue group) as compared to the control group (24.5% vs 19.5%, p = 0.01) which support the adhesion property of embryo glue as a transfer medium during embryo transfer by helping the embryo to adhere with endometrium lining into the uterus. A significantly increase live birth rate has been perceived in the test group as measured with the control group (80% vs. 65.4%, p = 0.005) which indicates that the use of embryo glue as a transfer medium can increase the live birth rate in per IVF cycle. There is a significant increase in an overall pregnancy rate in the test group (53.1% vs. 45.6%). There is significant decrease in the abortion rate in the test group was observed (20% vs 34.5%) which indicates that the use of embryo glue does not cause miscarriages at any stage of pregnancy. There is a significant increased multiple pregnancy rate was found in the test group (28.1% vs. 19%, p = 0.028) which states that embryo glue helps a maximum number of embryos to get implant which is transferred **(Fig. 4)**.

Further, when the comparison made between age groups, there is a significant increase in implantation rate of AMA age group of test arm observed (16.8% vs. 11.2%, p = <0.019) which specify that the embryo glue can be a beneficial tool to improve the implantation rate, and there is a significant increase in live birth rate of AMA age group (81.8% vs. 66.6%, p = <0.05) **(Fig. 5)**.

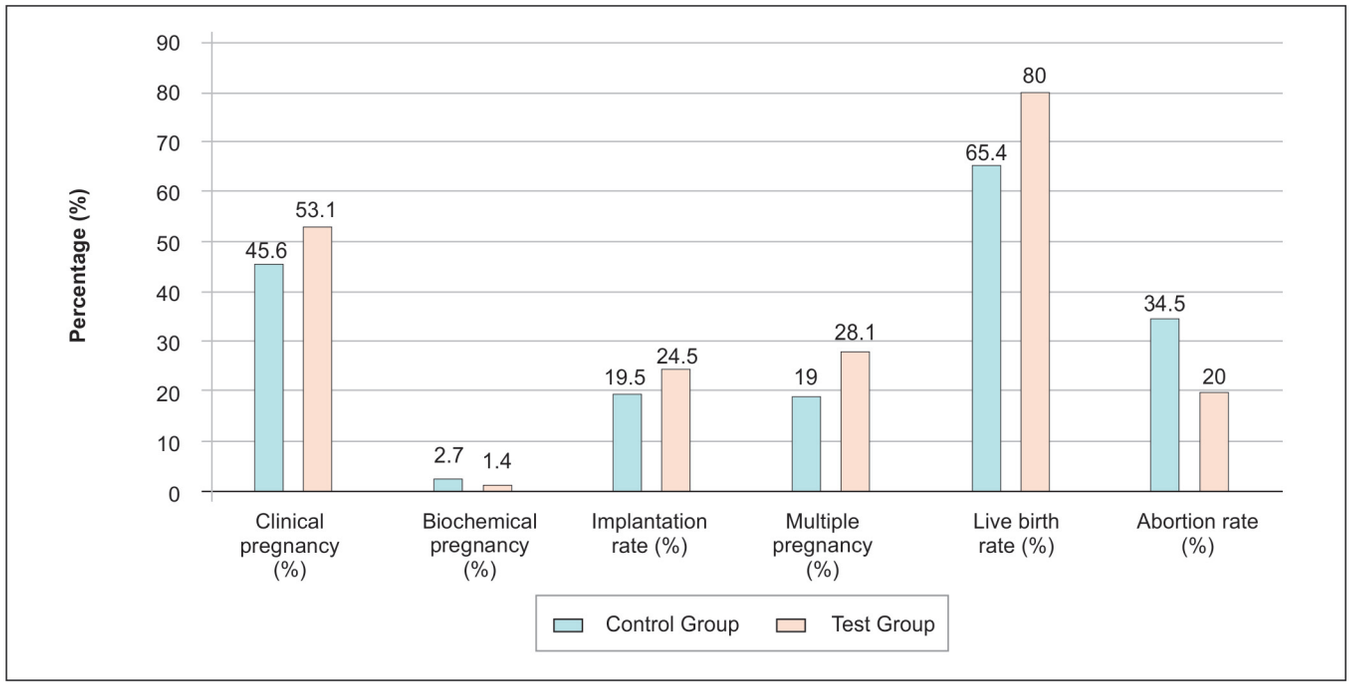


Fig. 4: Comparison of clinical outcomes between Test group (Embryo Glue) and Control group

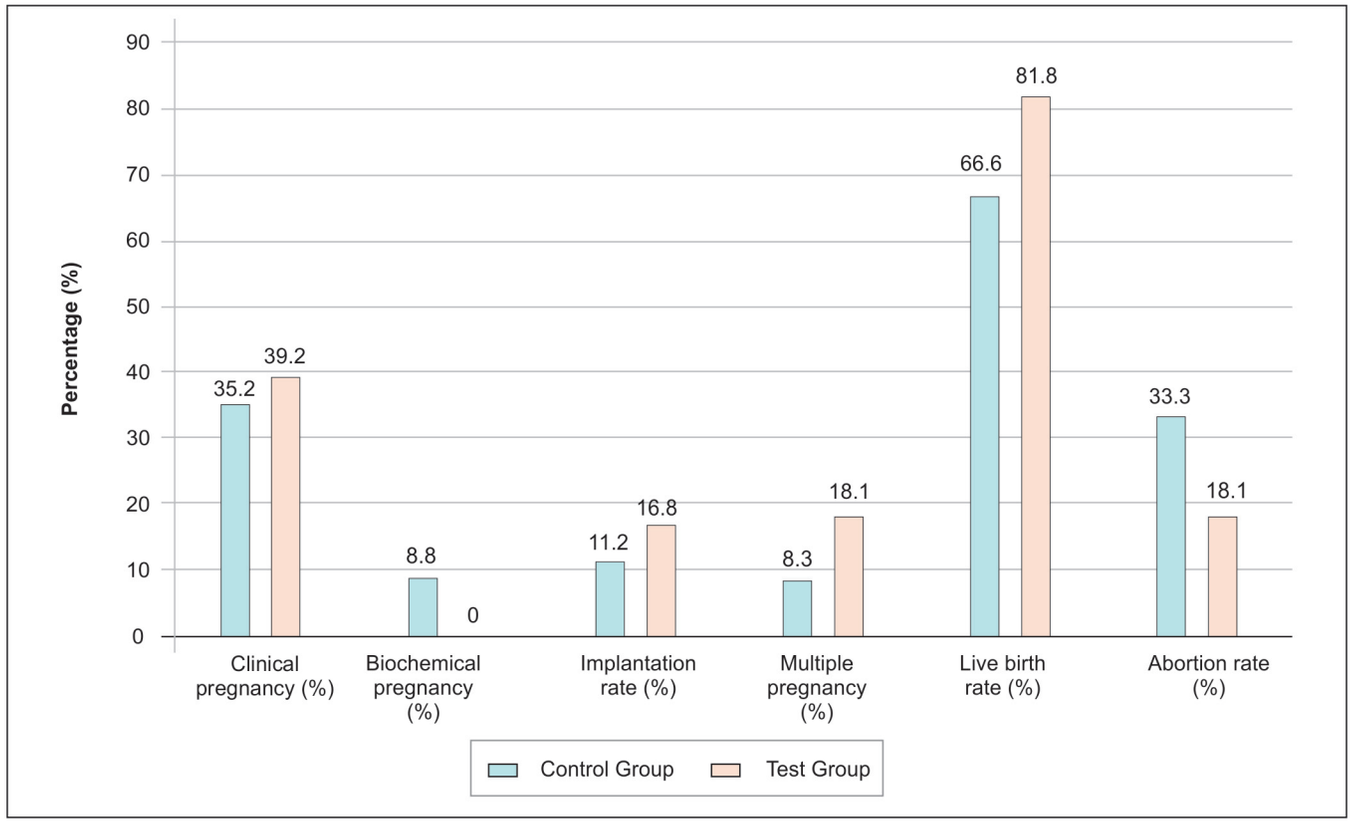


Fig. 5: Comparison of outcomes in AMA age group of test group and control group

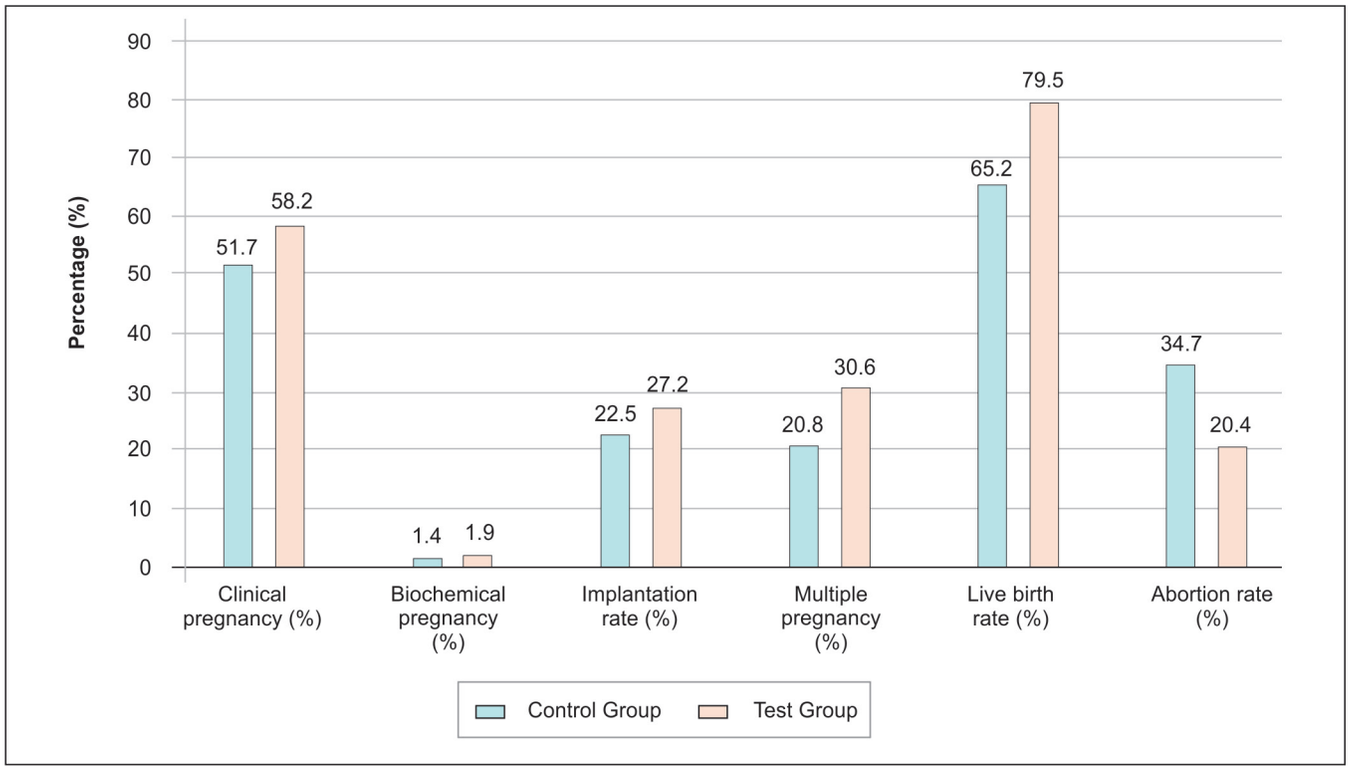


Fig. 6: Comparison of outcomes in young age group of Test group and control group

Therefore, in young age group patient, an increased value in all parameters have been observed which support the use of embryo glue for all those patients who are undergoing for FET with irrespective of age **(Fig. 6)**.

DISCUSSION

Implantation is a sensitive process that involves a complex interaction between the embryos and the endometrium lining of the uterus in the presence of many essential nutrient molecules and hormones secreted by the uterus lining.[4] Over the decades, important and noteworthy changes happened in the ART to achieve successful rates, however, implantation failure remains a major factor to deal with. Also, it is demonstrated by many authors in their study that with the increase of advanced maternal age, the outcomes of ART treatment decrease. Woman with AMA will have a poor ovarian response during controlled ovarian hyper-stimulation (COH) which eventually strikes the quantity and quality of oocytes, fertilization rate, good quality embryo yield, implantation rate, pregnancy rate and increase the incidents of miscarriage rate.

Gardner first reported in his study on the mouse model, that ET medium supplemented with a high hyaluronan concentration can significantly increase the rates of embryonic implantation and clinical results of IVF treatment.[14] Since then, several studies have also shown that hyaluronan incorporates a positive impact on the clinical outcome of embryo transfer. By observing the positive impact of higher concentration of hyaluronan in ET transfer medium, a commercial embryo transfer medium with higher amount of hyaluronan was prepared and launched in the market by Vitrolife.

Later on, after Gardner's study on HETM, the study published in favor of HA enriched transfer medium (HETM) by Schoolcraft et al. showed significantly higher implantation rates in human beings for the very first time.[12]

Hyaluronan is a glycosaminoglycan which is majorly present in several female reproductive organs like oviduct, uterine fluid, cervix, cumulus cells of the eggs, follicular fluid and seminal plasma as well.[15] The hyaluronan is synthesis by integral plasma membrane glycosyltransferases at the time of implantation and it is then exported directly into the extra cellular space.[16]

The action of hyaluronan is also known to be receptor mediated through binding to CD44 receptors. The binding process appears to be important for many physiological including embryonic development.[1]

In our study, we showed a significantly increased implantation rate (p = 0.01), Live birth rate (14.6% p = 0.005), overall pregnancy rate and multiple pregnancy (9.1% p = 0.028) rates in the HETM group of study (Embryo Glue) not only in AMA patients but in general. Also, decreased abortion rates were noticed in the HETM group as compared to the standard transfer medium.

In the study of Bulent urman et al.,[8] make an appearance that the enrichment of transfer medium with hyaluronan increase cumulative pregnancy rates and implantation rates in both embryo and blastocyst transfer. The favorable result was most evident in AMA, in women with poor quality embryos available for transfer and in women who had previous implantation failures.

Another study by Valojerdi et al.[9] reported upgraded clinical outcomes after the use of HETM. Although in 2014, a Cochrane review also suggested that although some moderate quality proof has shown supplement HA as an adherence blend in ART cycles could improve clinical pregnancy and live birth rates, more rigorous prospective studies require to be undertaken.[7]

A recent study by Neeta Singh et al. reported that it is difficult to conclude the favorable results of the utilization of embryo glue in IVF-ET cycles, however, in patient with recurrent implantation failure, it is often a useful.[5]

Lane et al. delineate that the incorporation of hyaluronan to the medium containing either bovine serum or recombinant albumin highly increase the flexibility of the blastocyst to survive cryopreservation.[11] Likewise, Stojkovic et al. reported that developmental capacity of bovine embryos in synthetic oviduct fluid substance hold either bovine albumin or hyaluronan. The addition of hyaluronan significantly increased the rapidity of blastocyst development.[13]

Hyaluronan may directly stimulate the development of the embryo or bear a more relevant environment for embryo to be nourished by supplements. Hyaluronan surface receptors were found on human and bovine embryos from the oocytes to the blastocyst stage. Hyaluronan may have a part in the assembling of the endometrium for embryo implantation as it increases significantly on the day of implantation in the mouse uterus and appears to be correlated with regions that contain stromal cells that are proliferating in devising for embryo implantation.[17]

HA is a viscous sticky component of the uterus. Because the uterine fluid is a viscous fluid, the transfer of a relatively aqueous mixture, such as culture medium with albumin or serum or hyaluronan, to the uterine lumen will affect in the slow dispersal of the medium and longer embryo adhesion with the luminal contents.

The studies suggest that, for better implantation, the interaction between the embryo-endometrium is important and our findings on the use of HETM in FET cycles were well explained. The HETM not only supports the implantation of the embryos but it enhances the growth of the embryo after transfer by providing cell adhesion, cell proliferation, cell migration, cell expansion, or losing.

Our findings suggest that the use of HETM to enhance the implantation rate in all groups of patients irrespective of age and cause of infertility is well defined and safe. In our study, we observed a significantly increased implantation rate, overall pregnancy rate, live birth rates and multiple pregnancy rate in the HETM group as compared to the standard transfer medium group. Also, decreased abortion rates were observed in the test group which showed no harmful impact of embryo glue on a later stage of pregnancy on the growing baby.

CONCLUSION

We can conclude that the use of hyaluronan-enriched transfer medium or embryo glue can be a safe and beneficial tool for patients undergoing treatment. Overall pregnancy rates and implantation rates are significantly higher by using HETM. To overcome the problem of multiple pregnancies, the number of embryos for transfer can be reduced. The beneficial effect of HETM on the patient with the age of >35 years is evidently shown in this study. The use of embryo glue in routine practice is safe and beneficial, Hence, the standard transfer medium can be replaced by embryo glue.

Source of Support

Nil

Conflict of Interest

None to declare.

Financial Disclosure

Nil

Acknowledgment

We acknowledge the doctors, colleagues who have supported us while conducting this study. We also acknowledge the patients who have participated.

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