CASE REPORT

Case Report: Effect of hyaluronic acid-enriched medium in case of post-menopausal *in vitro* fertilisation pregnancy

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**Abstract**

Hyaluronate-enriched medium has been gaining attention from researchers worldwide for its effectiveness in improving the success rate of embryo transfer following implantation in those patients who suffer from recurrent implantation failure. This article reports the case of a 49-year-old patient who was unable to get pregnant naturally, and with a history of three spontaneous abortions in her 20 years marriage period. The patient also had a history of four *in vitro* fertilisation (IVF) cycle failures before enrolling at Wardha Test Tube Baby Centre situated in Sawangi, India in August 2020. The patient had positive clinical pregnancy using conventional culture media devoid of hyaluronan instead of hyaluronan-enriched media. This case report enlightens the fact that embryo transfer may be unsuccessful using hyaluronan-enriched media in the case of post-menopausal patients.

**Keywords**

Blastocyst, Implantation, Glycosaminoglycans, Abortion, Conventional Media, Case Report

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Introduction
Infertility has long been reported as the inability of a couple to produce offspring despite repetitive unprotected sexual coitus for more than a year.1 To date, more than 180 million families have suffered infertility across the globe.2 It has been found that reproductive problems in women contribute approximately 37% of the reason for infertility among couples.3 Now, with advancement in technology, Assisted Reproductive Techniques commonly abbreviated as ART have come to aid the increasingly alarming situation as the infertility issues have started becoming more common around the world. Many researchers have pointed out to date that the secret to having positive pregnancy lies in the mechanism of implantation itself.4 There are different reasons for implantation failure such as diminished uterine wall receptivity, diminished quality of blastocysts, unsuitable condition of the uterus to support implantation of embryos etc.5 Also, several researchers have pointed out that the transfer media used for embryo culture during in vitro fertilisation (IVF) procedure also affects the rate of successful implantation.4,5 Nowadays, recent research has highlighted that the use of hyaluronic acid media can provide an increased positive effect on successful clinical pregnancy.4

Hyaluronic acid also referred to as hyaluronate and abbreviated as HA is an innately occurring glycosaminoglycan found in most of the tissues of the human body. It is unique to all other natural glycosaminoglycans because it is devoid of sulphur compounds and the site of formation of this macromolecule is also different to that of others. It is formed from two disaccharides (N-acetyl glucosamine and D-glucuronic acid).7 It also has a characteristic significant role played in our body such as helping in wound healing, cell proliferation, inflammation etc.7 The discovery of hyaluronic acid was in the year 1934 by Karl Mayer and John Palmer.7 Due to its wound-healing properties and hydration power, it is being used extensively in the cosmetic industry as well.7,9

Some researchers have studied the process of the menstrual cycle and it has been observed that throughout the luteal phase of the menstruation period, there is a presence of a higher quantity of hyaluronan and a slight drop of concentration at the onset of menstruation.7 It was the first time reported as a beneficial compound upon a successful clinical trial pregnancy of a mouse.4 Since then, several researchers have included this as a source of study to find a solution for improving the chances of successful clinical pregnancy in recurrent implantation failure cases.6 Commercially, for IVF procedures, hyaluronic acid is available under two types of media. One of the media is made up of a higher concentration of hyaluronate (0.5 mg/mL) along with a lower potency of albumin (2.5 mg/mL). In contrast to this, the other medium is made up of a lower concentration of hyaluronan medium (0.125 mg/mL) along with a higher concentration of albumin (10 mg/mL).4 This article highlights the effects of the use of different concentrations of hyaluronan medium in a case of post-menopausal clinical pregnancy to evaluate whether positive result is applicable to the post-menopausal infertile group.

Case presentation
Information related to the patient
This case report has been studied on a couple who visited Wardha Test Tube Baby Centre located at Sawangi, India (part of Datta Meghe Institute of Higher Education and Research) who came to seek treatment for their infertility in August 2020. A 49 year old South Asian female patient who was diagnosed with secondary infecundity for approx. 19 out of 20 years of her married life was enrolled for four cycles of IVF treatment. The patient was post-menopausal for four years when she visited the centre to treat her infertility. The patient’s menstrual cycle was regular during her menstrual years and menstrual loss was average. The South Asian male partner, aged 53 years, was a jeweller by profession along with farming activities and the female partner’s work history was as a homemaker. Both the patient and her male partner did not have a history of alcohol consumption, tobacco consumption, or any regular habit of smoking.

Medical history of the couple
The case study revolves around a female who had a medical history of spontaneous abortion thrice in her married life. Also, she had a history of galactorrhoea. The patient had menarche at the age of 14. They had no sexual problems in their marriage and had faced secondary infertility for 19 years in their marriage period of 20 years. Upon doing a semen analysis of the male partner as a part of the treatment, it was reported normal; the total count of sperm in the semen sample was 50 million per ml and the percentage of motile sperm in the semen sample was 70%. The morphologically good quality sperm was found to be 7%. The husband had no history of taking any medicines prior to their visit at centre for treating their infertility.

The patient had a medical history of hyperpiesis and hypothyroidism in the last 2 years. She was undertaking captopril and thyroxine supplement tablets of 1.50mcg each per day for the treatment of the diseases respectively in the past 2 years. Other than that, the couple’s family medical history reportedly didn’t have any conditions like diabetes mellitus, asthmatic conditions, psychotic diseases or any major surgeries. The patient had undergone intrauterine insemination (IUI) and intra-cytoplasmic sperm injection followed by embryo transfer (ICSI-ET) four times with the semen of the
husband four years before visiting Wardha centre. All attempts of IUI and ICSI-ET failed in this patient prior to their visit at Wardha Test Tube Baby Centre.

**Patient’s medical history**

For a successful program transfer of higher-quality embryos, the normal value of oestrogen should be around 300 to 500 pg/mL.\(^{10}\) and serum progesterone should be below 20 ng/mL.\(^{11}\) The thyroid stimulating hormone value for infertile patients should be below 2.5. It has been reported that elevated or diminished levels of thyroid hormone in female patients hinder the chances of conception. The hormonal levels in the patient upon testing in the clinical laboratory of Wardha Centre were found to be: oestradiol-412 pg/mL, progesterone-17.1 ng/mL, and thyroid stimulating hormone (TSH)-3.34 μIU/ML. Anti-mullerian hormone (AMH) is also an important criterion to gauge the ovarian reserve of patient which directly affects the follicular count. A higher value of AMH denotes a higher number of oocyte presence and higher ovarian reserve. In this patient, the value of the Anti-mullerian hormone was found to be 1.5. The value of AMH shows that the patient has a normal ovarian reserve. Antral follicular count (AFC) is also a predictable asset for ovarian reserve and also to analyse the size of the remaining primordial follicular pool. The patient reported having good antral follicular count (AFC).

Due to menopause and the age of the patient, her ovaries were unable to produce oocytes for the treatment. Hence, the embryos were formed by using donor oocyte and the sperm used was that of the husband. The patient had been counselled for direct transfer of embryos. In the first cycle of the IVF treatment, we opted for fresh embryo transfer. She was started with the tablet Estradiol (E2) 2 mg twice a day on a daily routine and vitamin supplements along with thyrroxine supplement tablet of 150 mcg once a day as pre-treatment medication. Day five embryo quality was assessed and two, grade I fresh embryos was transferred to the patient. These embryos are referred to as blastocysts and are of best quality as per Gardner blastocyst grading system.\(^{12}\) The embryos were transferred without any obstacles in August 2020. This was her fifth embryo transfer and first attempt at our centre. Testing of \(\beta\)-hCG was undertaken after 14 days of embryo transfer in a clinical laboratory located in Sawangi, India. The result showed as negative. The patient was counselled upon the following situation and wished to try again. On subsequent transfers, only frozen embryos were used for transfer in the patient. In the second IVF treatment cycle, we experimented with the transfer of embryos on day four, but the results were negative. There were four IVF treatment cycles performed on the patient using different techniques. In the third IVF treatment cycle, we experimented on the transfer of day three frozen embryos, but it also led to a negative test result. On the fourth cycle of IVF treatment on February 2022, three day five embryos were assessed and frozen for further use. During each follow-up, we analysed the denseness of the endometrium which is a key parameter for the successful implantation of the embryo in the uterus. The wideness of the endometrium should be below 5 mm for starting the medication for the preparation of the transfer of embryos. The patient was put on medications with the tablet Estradiol (E2) 2 mg two times a day on a daily basis and vitamin supplements along with thyrroxine supplement tablet of 150 mcg once a day as pre-treatment medication.

Embryo thawing was performed by using a worldwide accepted thawing kit and was stored in Benchtop Incubator for around 120 minutes while the embryos expand. The embryos were kept to be thawed for two hours. Once, it was successfully achieved, two embryos of day five which constituted 4AA and 4AB grades (Figure 1) were passed on into the uterus of the patient. The embryos were transferred without issue in May 2022.

**Follow-up diagnostic assessment and therapeutic advises**

After the transfer of the embryo is successfully made, a follow-up is requisitely advisable to the patient. A consistent follow-up is advised up to 2 weeks starting from the day of transfer of embryo. Regular medications were prescribed to the patient. The patient was advised to have oestradiol 2 mg twice a day, aspirin 75 mg once a day, multivitamins and iron tablet once a day, prednisolone once a day, dydrogesterone tablet once daily, progesterone 400 mg once a day, famotidine 40 mg once a day empty stomach. She was also advised injectable medication of human chorionic gonadotrophin (HCG), filgrastim, enoxaparin 40 mg, hydroxyprogesterone caproate 500 mg and intralipid injection. On the 14\(^{th}\) Day, the blood sample was collected from the patient to test for \(\beta\)-hCG and it returned a positive outcome. The level of \(\beta\)-hCG value was found to be 648 mIU/ml. Upon careful observation, we could find that out of the two embryos that were transferred, the 4AA embryo was successfully implanted into the walls of the uterus.

**Discussion**

Heymann et al. (2020) studied the effect of hyaluronic acid on implantation rate and found positive result on it.\(^{13}\) Also, Ganji et al. (2022) analysed the effect of hyaluronic acid on implantation rate and found similar result.\(^{14}\) These articles formed the strength of this case report. The limitations associated with this case report is that the result of this report alone cannot be applied on post-menopausal group patients and requires further randomised clinical trials to analyse the result on larger sample population and application to post-menopausal group. It has been an important and interesting matter of
research for many scientists to find the various reasons behind the failure of implantation. There are many reasons such as advanced maternal age, uterine conditions such as a uterine polyp, sub-optimal quality of egg formation, elevated basal metabolic index, stress etc.\textsuperscript{15} It has been also observed that despite suitable conditions for the transfer of embryos, implantations have failed. They have been categorized as idiopathic cause of implantation failure. In our case study, the patient has been suffering from infertility and unexplained abortion due to unknown reasons, despite having no major medical alignment during her fertility years. Hence, we sought to find out the possible cause behind the failure of implantation and therefore, unsuccessful pregnancy attempts. The patient was also under the category of advanced age and post-menopausal group, hence successful IVF procedure was a challenge in itself. In the post-menopausal phase, the female body does not produce a lot of essential hormones and components like hyaluronic acid that are required for a successful pregnancy. It has been reported that the use of a hyaluronic-enriched medium can have a favourable sequel on the chances of pregnancy.\textsuperscript{4}

The implantation success is also dependent on various factors like the quality of embryos, endometrial receptivity, positive embryo-uterine interaction etc. Implantation is termed as successful upon the formation of weaker apposition in the blastocyst which is the requisite criteria for fixation at the wall of the endometrium which is usually followed by invasion into the endometrial stroma.\textsuperscript{7} However, despite the necessary modification of the embryo, it has been observed that a successful transfer has failed due to unknown reasons. Despite the known literature reasons that lead to failure of implantation such as genetic factors, the inability of the embryo to undergo necessary modifications and lower receptivity in the uterine lining, there are other causative factors as well which equally contribute to successful implantation.\textsuperscript{16}

It has been published recently by one of the research projects that the use of adherence compounds such as hyaluronic acid can contribute to positive pregnancy outcomes in assisted reproductive techniques.\textsuperscript{4,13} Hyaluronic acid also known as hyaluronan is an anionic molecule made up of non-sulphated glycosaminoglycans which are found in almost all tissues of the human body. It is a special type of glycosaminoglycan as it is devoid of sulphate compounds and it formed mostly in the plasma membranes of the body instead of the typical location - Golgi bodies. Its major contribution lies in the healing of cell injuries and also helps in cell proliferation.\textsuperscript{7,9} It is this feature that attracted many researches and they have experimented with incorporating it into IVF procedures.\textsuperscript{4,13} The transfer media that we used for embryo culture was experimented with by infusion of hyaluronic acid. Recent studies show that the addition of hyaluronic acid-enriched transfer media for embryo transfer has shown a considerable increase in successful implantation as compared to transfer media which were devoid of hyaluronic acid.\textsuperscript{4,13}
Also, previous studies have reported that there are no negative adverse effects like miscarriages, multiple births, or gestational changes upon the use of the hyaluronan-enriched medium. Studies have also highlighted the null effect as an attachment sealant upon the application of hyaluronate media. In our case study, the patient had undergone four IVF cycles which included one attempt of fresh transfer of embryos and three attempts of frozen transfer of embryos. The transfer media in which the embryos were cultured has experimented with the addition of different concentrations of the hyaluronan enriched medium along with one cycle which was devoid of the addition of this non-sulphated glycosaminoglycan and conventional culture media was used, based on the research that stated hyaluronan acid enriched medium improves successful implantation. The different concentrations of hyaluronan acid media that were used are Sample I medium, which contained a lower concentration of hyaluronan acid (0.125 mg/mL) and Sample II medium, which contained a comparatively higher concentration of hyaluronan acid (0.5 mg/mL).

However, the result that we concluded based on the study, stated otherwise. We found in our case study that despite the use of hyaluronate-enriched media, both the procedure of transfer of fresh embryos and the transfer of frozen embryos resulted in a negative β-hCG test. Upon no use of a hyaluronan acid-enriched medium in our fourth IVF procedure, the patient resulted in a positive β-hCG report.

**Conclusions**

This case report is a testimonial of a post-menopausal patient achieving successful clinical pregnancy outcome through assisted reproductive technique after eight attempts of IVF procedure (four attempts of failed IVF procedure as medical history before visiting Wardha Test Tube Baby Centre for seeking assistance in their fertility journey). This case also highlights that use of a hyaluronan acid-enriched medium may not have a significant effect in the case of post-menopausal successful clinical pregnancy.

**Consent**

Written informed consent for publication of their clinical details and clinical images was obtained from the patient and her husband.

**Data availability**

All data underlying the results are available as part of the article and no additional source data are required.

**References**

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