

Article

Does Vaginal Delivery of Concentrated Oxygen and Hyaluronic Acid Improve the Implantation Rate in Human IVF: A Proof-of-Concept Study

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Abstract

Objective: To determine whether a high concentration of oxygen and hyaluronic acid applied vaginally in the two weeks preceding embryo transfer may increase implantation rates in human IVF. **Design:** Retrospective, observational, non-randomized proof-of-concept study. CaressFlow treatment consisted of three 15 min vaginal sessions administered during the two weeks preceding embryo transfer, using oxygen at $93\% \pm 3\%$, a pressure of 1 ± 0.1 bar, a flow rate of 2 ± 0.2 L/min, and 0.2% sodium hyaluronate. **Subjects:** A total of 377 women with infertility and/or previous IVF failure undergoing single frozen-thawed blastocyst transfer were included. Of these, 161 received CaressFlow treatment and 216 served as untreated controls. **Main Outcome Measures:** Implantation rates after homologous and heterologous frozen blastocyst transfer. **Results:** Overall, implantation occurred in 94 of 161 treated patients (58.4%) compared with 92 of 216 controls (42.6%), corresponding to significantly higher odds of implantation in the CaressFlow group (OR 1.89, 95% CI 1.25–2.86; $p = 0.0024$). In the homologous subgroup, implantation occurred in 60 of 111 treated patients (54.1%) and 64 of 147 controls (43.5%); this difference was not statistically significant (OR 1.53, 95% CI 0.93–2.50; $p = 0.0941$). In the heterologous subgroup, implantation occurred in 34 of 50 treated patients (68.0%) compared with 28 of 67 controls (41.8%), corresponding to significantly higher odds of implantation following CaressFlow treatment (OR 2.96, 95% CI 1.37–6.38; $p = 0.0050$). **Conclusions:** In this retrospective proof-of-concept study, vaginal pretreatment with high-concentration oxygen and hyaluronic acid was associated with higher implantation rates following single frozen-thawed blastocyst transfer. The association was most pronounced in patients undergoing heterologous embryo transfer. Prospective randomized studies are required to confirm these findings and to clarify the underlying mechanisms.

Keywords: high concentrations of oxygen and hyaluronic acid; vaginal delivery; pre-transfer; frozen blastocyst transfer; implantation rates; homologous; heterologous



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1. Introduction

Implantation failure following assisted reproductive techniques often remains an unresolved dilemma. Although embryo culture has improved notably over the past two decades, there have been fewer advances in understanding endometrial receptivity. It has been estimated that embryo viability accounts for one third of implantation failures, while sub-optimal endometrial receptivity and altered embryo-endometrial dialog

are responsible for the remaining two thirds [1–3]. Even following blastocyst selection using pre-implantation genetic diagnosis for ploidy, the implantation rate hovers around 40–60% [4]. Embryo implantation in humans is a complex orchestration of complementary tissues, involving a temporal sequence of dialog and depends on an oxygen- and nutrient-rich uterine environment [5].

Since the classical work of Rock and Bartlett [6] describing the histological changes in the endometrium at the time of implantation, there have been many attempts to improve the efficiency of implantation. Protocols for the timing of transfer of in vitro human embryos revolve around monitoring the endothelial lining with ultrasound [7]. A wide variety of pharmaceutical options to improve implantation rates exist and range from hormonal therapy, through cortisone to vasodilators. Specific attempts to identify the window of implantation have seen the introduction of the ERA test (Endometrial Receptivity Array), where several hundred molecular markers have been suggested to be indicative of a receptive endometrium [8]. To date, the ERA test is considered to be of limited clinical use [9]. Mechanical stimulation of the uterine lining by endometrial scratching has been used for decades in an attempt to improve the implantation rate [10], while much attention has been given to immune alterations and therapies in human endometrial receptivity [11]. Attempts to improve endometrial receptivity by infiltration of platelet-rich plasma (PRP) have also recently been adopted [12], while the elasticity of the cervix has been considered as a marker for ease of transfer and improved implantation rates [13]. Uterine contractility at the time of embryo transfer, cervical stenosis, and indeed the adequacy of the embryo transfer technique may all lead to negative outcomes after assisted reproduction [14].

An equilibrated vaginal microbiome has been used as a marker to indicate a favorable implantation platform [15], and recently it has been shown that the vaginal biome may be significantly altered following erogation with high concentrations of oxygen and hyaluronic acid [16], with marked improvements in hydration of the vaginal epithelia. This minimally invasive technology is now widely used to treat a variety of vaginal and vulval pathologies, including lichen, atrophy, urethral pain, candidiasis and vaginal dryness [17–19].

More recently, it has been demonstrated that microbial contamination of the embryo transfer catheter with cervicovaginal microorganisms, other than *Lactobacilli*, can significantly reduce pregnancy and live birth rates in an IVF program [20].

The present study is a pilot, proof-of-concept, retrospective, observational, case-controlled study to determine whether high O₂ and hyaluronic acid applied vaginally in the two weeks preceding an embryo transfer may increase implantation rates. Frozen–thawed blastocysts were used in single embryo transfers in 377 patients from CFA-Italy, Naples.

2. Methods

Couples attending CFA-Italy gave written consent for this preparatory treatment. Those enrolled met the following criteria: infertility, defined as failure to conceive after 12 months and/or previous IVF failures. Patients with a previous history of cervical diseases, cervical surgery or infection were excluded. Male gametes were from partners with normospermia, various degrees of oligastenoteratospermia and also sperm extracted from Tese samples. Female partners were stimulated with gonadotrophins to increase the number of oocytes superovulated. Oocyte collection, fertilization, embryo culture and blastocyst vitrification were carried out by standard procedures (Homologous). All homologous patients received frozen–thawed blastocysts. Other patients received a frozen–thawed blastocyst from an oocyte donor using the husband’s spermatozoa (Heterologous). The treated group differed from the control group in the application of high vaginal oxygen and hyaluronic acid. A self-cooling Caress Flow (Caress Flow srl, Casette di Fumo, Bologna, Italy) device was used to apply high O₂ (93% ± 3%) at a pressure of 1 ± 0.1 bar at a

flow of 2 ± 0.2 L/min containing 0.2% sodium hyaluronate in three 15 min sessions in the two-week period prior to embryo transfer. The first 14 days before embryo transfer, the second 7 days before transfer, the last 24 hrs before transfer. Synchronization and preparation of the uterus followed a standardized protocol for both the control group ($n = 216$) and the Caressflow-treated group ($n = 161$). Three hundred and seventy-seven patients attending CFA in Naples from September 2024 until April 2025 were used in this study and assigned either to the treated group ($n = 161$) or the control group ($n = 216$). The average age in the treated group was 38.0 years, while the average age in the control group was 37.5 years.

Laboratory conditions and blastocyst quality were comparable in the two groups, as were embryo transfer protocols. In this study, we have not included blastocysts that had undergone chromosomal screening (PGT-A), as this add-on is not requested by many patients. Serum β hCG was measured 9 days after embryo transfer, with a value of over 90 mIU/mL considered positive.

3. Statistics

Statistical analyses were performed using R software (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria) within the RStudio environment. Implantation outcome was analyzed as a binary variable (successful vs. unsuccessful implantation). Comparisons between the CaressFlow-treated and control groups were performed for the overall study population and separately for the homologous and heterologous embryo-transfer subgroups. For each comparison, a 2×2 contingency table was constructed using the numbers of successful and unsuccessful implantations. Differences between groups were assessed using Pearson's chi-squared test. The assumptions for this test were satisfied in all comparisons, with expected cell frequencies greater than 5. Treatment effects were expressed as odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs). All statistical tests were two-sided, and a p -value < 0.05 was considered statistically significant. Statistical results were graphically summarized using implantation-rate bar plots and a forest plot displaying ORs and corresponding 95% CIs.

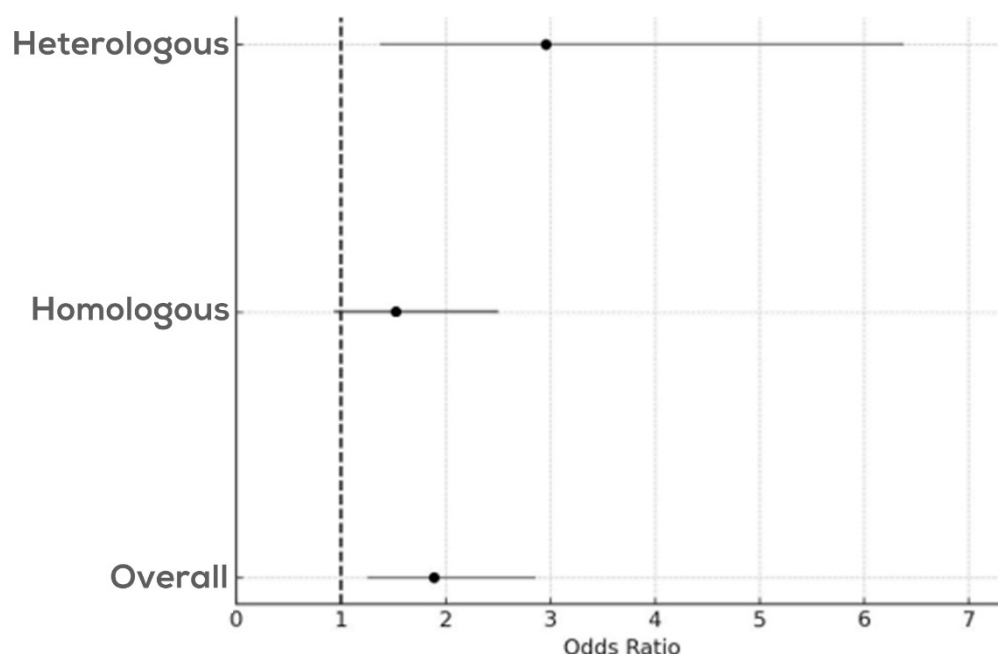
4. Results

Overall, implantation was successful in 94 of 161 patients (58.4%) in the CaressFlow-treated group compared with 92 of 216 patients (42.6%) in the control group. CaressFlow treatment was therefore associated with significantly higher odds of implantation in the overall population (OR 1.89, 95% CI 1.25–2.86; $p = 0.0024$).

When patients were stratified according to the type of embryo transfer, implantation occurred in 60 of 111 patients (54.1%) in the treated homologous subgroup and in 64 of 147 patients (43.5%) in the corresponding control subgroup. Although the implantation rate was numerically higher following treatment, the difference did not reach statistical significance (OR 1.53, 95% CI 0.93–2.50; $p = 0.0941$).

In the heterologous subgroup, implantation occurred in 34 of 50 treated patients (68.0%), compared with 28 of 67 control patients (41.8%). This difference was statistically significant, corresponding to approximately three-fold higher odds of successful implantation in CaressFlow-treated patients (OR 2.96, 95% CI 1.37–6.38; $p = 0.0050$). Implantation rates and effect-size estimates for all comparisons are summarized in Figure 1A and Figure 1B, respectively.

A-Odds ratios for successful implantation



B-Implantation rates by treatment group

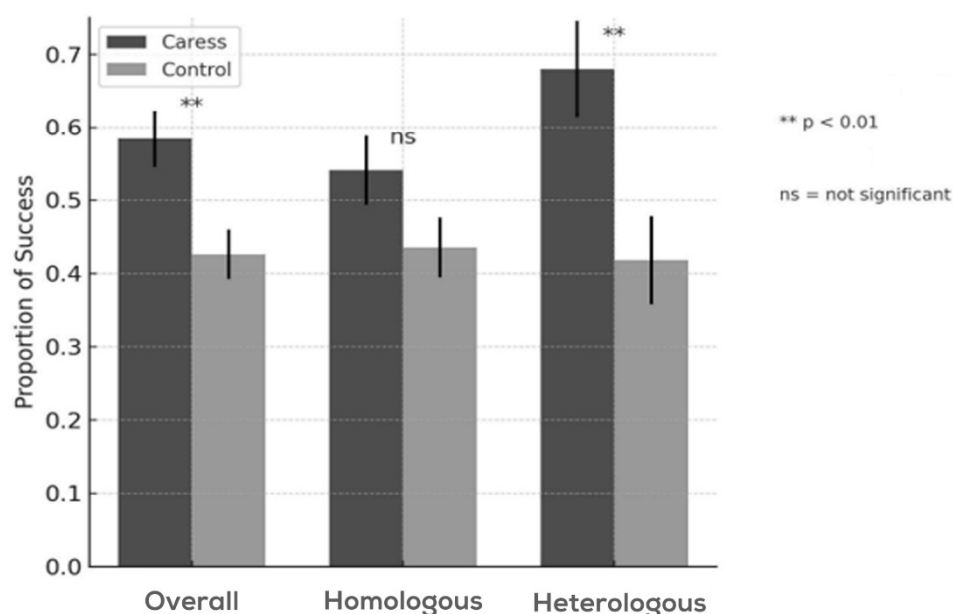


Figure 1. Effect of CaressFlow treatment on implantation outcome following single frozen–thawed blastocyst transfer. **(A)** Forest plot showing the odds ratios (ORs) for successful implantation in CaressFlow-treated patients compared with the corresponding control groups in the overall population and in the homologous and heterologous embryo-transfer subgroups. Dots indicate the estimated ORs and horizontal lines represent the corresponding 95% confidence intervals (95% CIs). The vertical dashed line at OR = 1 indicates no difference between CaressFlow treatment and control. **(B)** Implantation rates in CaressFlow-treated and control patients in the overall population and in the homologous and heterologous embryo-transfer subgroups. Bars represent the proportion of successful implantations. Statistical significance of the comparison between treated and control patients is indicated above each pair of bars (** $p < 0.01$; ns, not significant).

5. Discussion

Implantation of the human embryo is a complex dynamic interaction of complementary tissues [5]. Formation of a rapidly expanding blastocyst cavity is a prerequisite to hatching and depends, in part, on the adequate expression of gap junctions, tight junctions and desmosomes [21,22]. The trophoblast cells produce proteolytic enzymes to hatch free of the protective zona pellucida, and these may be complemented by proteolytic enzymes produced by the uterus. A molecular dialog between the hatched blastocyst and the endometrium favors attachment to the epithelium. The blastocyst trophoblast then secretes hCG, which signals the epithelial cells, displacing them and allowing the embryonic cells to penetrate the basement membrane. Thus, for implantation, we may consider three phases: apposition, adhesion and invasion, which must occur at a specific time, i.e., when the uterine epithelium is receptive, “the implantation window”.

This window of opportunity is thought to be less than 24 h long in humans and occurs approximately six days after ovulation [5]. Attempts to identify this window by biopsy (ERA test) have fallen short [9,23]. The blastocyst initially derives nourishment from uterine secretions, but in order to keep growing it must develop its own vascular system. Maternal hormones influence communication between the embryo and the endometrium, with cytokines, adhesion molecules, prostaglandins, metalloproteases and angiogenic growth factors all involved.

In this study, we have used as a baseline frozen–thawed blastocysts and, while maintaining patients’ personalized therapies for individualized endometrial preparation, our primary aim was to determine whether the application of high concentrations of vaginal O₂ and hyaluronic acid could alter the implantation rate in a trial with 377 patients. It is the policy of CFA-Italy to offer PGT-A (evaluation of ploidy) only to patients who request this add-on, and we do not routinely screen for chromosomal number in donor embryos. It would be interesting to carry out a further study using PGT-A screened blastocysts to determine whether the implantation rate could be further increased. However, we would like to point out that the control implantation rates in the homologous and heterologous sub-groups are similar, pointing to an intrinsic similarity in embryo implantation capacity. This is supported by the fact that the heterologous sub-group received donated embryos from younger ovarian sources than the homologous sub-group. In our study, we have shown that there is a significant increase in implantation rate in the Caress flow-treated population, with a highly pronounced difference in the heterologous population. Assuming that the blastocysts from the treated group and the Caressflow-treated group have similar characteristics, the main difference between the heterologous and homologous populations is the age of the receiving patient and probably reflects the inherent differences in uterine physiology of the two patient groups. More studies are required to determine age-related changes in uterine receptivity.

Considering the multi-factorial process of early human reproduction, it is feasible that the effects of our combined O₂ and hyaluronic acid treatment protocol are also multiple. The reasons for the improved implantation rate in our study at this point can only be the object of conjecture. Our study has not determined whether Oxygen or Hyaluronic acid alone may have led to the increase in implantation rate or whether the two molecules must act synergistically. Briefly, we would like to raise interest in two simple, yet fundamental, pathways. One major factor to consider is the pH of the uterine environment, which appears to be around pH 7.7 in non-pregnant women [24]. Studies on other mammals also point to an alkaline uterus with changes correlated with the oestrus cycle in the rat and sow [25,26]. A study is in progress at CFA to measure uterine extracellular pH during the oestrous cycle in normo-cycling women. The human oocyte and early cleavage embryo have a p*H*_i of 7.4 and have poor tolerance to extracellular pH insults below 7.0; however,

on Day 5 the human blastocyst acquires acid-regulating pumps on the plasma membrane and is able to tolerate both acid and alkali conditions [27]. Intracellular pH will, of course, influence all cellular processes. Furthermore, it has been shown that the contractility of the human myometrium is altered by intracellular pH [28] and that the very low vaginal pH of around 4.0 in cycling women is due to the presence of *Lactobacillus* species. Considering that high concentrations of O₂ and hyaluronic acid are known to improve the vaginal microbiome, increase the hydration of the vaginal mucosa and improve the vascularity of the cervix [16], it would be of interest not only to study the uterine pH but also to classify the uterine microbiome before and after treatment. In fact, in a recent study it has been shown that implantation rates may be substantially reduced by the microbial contamination of the embryo transfer catheter with cervicovaginal microorganisms [20].

Although at present we have no direct evidence that vaginal application of O₂ will influence the uterine cavity, a second pathway related to high concentrations of O₂ to be considered is its role in driving redox-signaling and, in particular, stimulation of expression of growth factors, such as VEGF, reducing inflammatory processes and promoting angiogenesis [29,30]. Similarly, we do not know if the vaginally applied Hyaluronic acid may migrate to the uterus; however, we should point out the extensive yet controversial literature on the use of HA as an embryo glue, i.e., its use in the transfer medium to enhance adhesion and implantation [31].

Finally, since it is known that implantation rates are dependent on the adequacy of the transfer technique [13], we raise the possibility that an improvement in cervical physiology may also account in part for the significant increase in implantation rate.

6. Conclusions

Our pilot retrospective study on a population of 377 infertile patients treated at CFA, Naples, from September 2024 to April 2025 demonstrates that implantation rates of frozen–thawed blastocysts may be significantly improved by the vaginal delivery of high concentrations of oxygen and hyaluronic acid in the two-week period preceding embryo transfer. The most significant increase in implantation rate was observed in patients receiving donated blastocysts. We discuss the possible reasons for this increase, which may include changing the vaginal and uterine microbiome, altering endometrial extracellular pH, or cervical physiology.

Author Contributions: B.D., R.F., D.D., M.D., C.R. and A.R. conceived and designed the study. F.G. analyzed the data. B.D. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study protocol was approved by the Ethical Committee of the University of Naples (CET3), protocol number 95/2026, and was performed in accordance with the Declaration of Helsinki. All patients signed an informed consent form. Caressflow is a European-approved medical device for applying a high concentration of O₂ and hyaluronic acid vaginally (CE IT293213-1).

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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