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Effect of embryo transfer catheter incubation strategy on pregnancy and live birth outcomes: a retrospective comparative cohort study

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Abstract

Background

Embryo transfer is one procedural step that can influence assisted reproductive technology outcomes. Although embryos are cultured under controlled conditions, loading and transfer may expose the embryo-containing catheter to transient cooling. This retrospective comparative cohort study assessed whether the recorded embryo transfer catheter incubation strategy was associated with pregnancy and live birth outcomes.

Methods

Women aged 21–40 years who underwent fresh or frozen-thawed embryo transfer at a tertiary assisted reproduction centre in Goa, India, between January 2024 and July 2025 were included if complete outcome follow-up was available. Cycles were categorized according to the catheter handling protocol documented in the embryology record: inner catheter incubation only (Group A), outer sheath-only incubation (Group B), or combined inner catheter plus outer sheath incubation (Group C). Catheter components assigned to incubation were placed in a CO2 incubator at 37°C for at least 15 min before embryo loading. Primary outcomes were clinical pregnancy and live birth per embryo transfer cycle.

Results

A total of 240 embryo transfer cycles were analyzed, with 80 cycles in each group. Baseline demographic, clinical, and embryological characteristics were similar across groups. Clinical pregnancy occurred in 42/80 cycles (52.5%) in Group A, 28/80 cycles (35.0%) in Group B, and 47/80 cycles (58.8%) in Group C (P = 0.008). Live birth occurred in 35/80 cycles (43.8%), 22/80 cycles (27.5%), and 40/80 cycles (50.0%), respectively (P = 0.011). Compared with outer sheath-only incubation, the absolute live-birth difference was 16.3 percentage points for inner catheter incubation and 22.5 percentage points for combined incubation. After adjustment for maternal age, embryo stage, transfer type, endometrial thickness, number of embryos transferred, and transfer of at least one top-quality embryo, inner catheter incubation (adjusted odds ratio 1.97, 95% CI 1.02–3.82) and combined incubation (adjusted odds ratio 2.56, 95% CI 1.31–4.98) remained associated with live birth.

Conclusions

In this multi-centre retrospective cohort, incubation of the embryo-carrying inner catheter, either alone or with the outer sheath, was associated with higher clinical pregnancy and live birth rates than outer sheath-only incubation. The findings support prospective evaluation of catheter warming protocols and direct temperature monitoring before routine protocol adoption.

Background

Embryo transfer (ET) is the final clinical step of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment. Advances in stimulation, vitrification, embryo culture, and embryo selection have improved assisted reproductive technology (ART) practice, but implantation failure remains common. The transfer procedure is therefore an important point at which laboratory conditions and clinical technique meet, and small procedural differences may affect the chance of implantation and live birth [1].

The preimplantation embryo is handled in a tightly controlled laboratory environment, with attention to temperature, pH, gas phase, and culture medium stability [2, 3]. Embryo morphology assessment is also standardized to reduce variability in embryo selection and reporting [5]. Despite these controls, the brief interval between embryo loading and intrauterine deposition can expose the embryo-loaded catheter to room temperature. Experimental work has shown that cooling can disturb temperature-sensitive cytoskeletal structures in gametes [6], and simulated ET studies have demonstrated rapid temperature decline within loaded transfer catheters during routine handling [7, 8].

Soft ET systems typically include an outer sheath that guides uterine access and an inner catheter that carries the embryo-containing medium. In routine practice, some laboratories warm only the inner catheter, some warm only the outer sheath, and some warm both components. Existing ET guidance supports standardization, ultrasound guidance, atraumatic transfer, and soft catheter use, but it does not define a preferred approach to component-specific catheter incubation [1].

The present study compared three catheter handling strategies used in routine care: inner catheter incubation only, outer sheath-only incubation, and combined inner catheter plus outer sheath incubation. The objective was to determine whether the recorded catheter incubation strategy was associated with clinical pregnancy and live birth after fresh or frozen-thawed ET.

Methods

Study design and setting

This was a retrospective comparative cohort study conducted across tertiary ART centres in Goa, India. Embryo transfer cycles performed between January 2024 and July 2025 were reviewed after completion of clinical outcome follow-up. The study compared three catheter handling protocols documented in routine embryology and clinical records.

The study was approved by the Institutional Ethics Committee of Directorate of Health Services, Goa [approval number: DHS/Sp-Cell/24–166(Ethics)/2025-26/1208, dated 19/08/2025]. The study used de-identified routine clinical records therefore, the requirement for individual written consent for this retrospective analysis was waived by the approving committee. All procedures were conducted in accordance with the Declaration of Helsinki and applicable Indian ART regulations [10, 11].

Participants and eligibility criteria

Women undergoing fresh or frozen-thawed ET were eligible for inclusion. Inclusion criteria were female age 21–40 years, transfer of at least one viable day 3 cleavage-stage embryo or day 5/6 blastocyst, and a normal uterine cavity confirmed by hysteroscopy or transvaginal ultrasonography within the preceding 3 months.

Cycles were excluded in the presence of untreated hydrosalpinx, intrauterine adhesions, submucosal fibroids distorting the uterine cavity, congenital uterine anomaly affecting embryo placement, endometrial thickness less than 6 mm on the day of transfer, donor oocytes, donor sperm, surgically retrieved sperm, preimplantation genetic testing cycles, difficult or traumatic mock transfer requiring protocol deviation, or incomplete outcome follow-up.

Exposure groups: catheter handling strategy

Cycles were categorized according to the catheter handling strategy recorded at the time of transfer. No random allocation was performed.

Group A, inner catheter incubation: the sterile inner embryo transfer catheter was placed in a CO2 incubator at 37°C for at least 15 min before embryo loading. The outer sheath was maintained at room temperature according to routine sterile preparation.

Group B, outer sheath-only incubation: the sterile outer sheath was placed in a CO2 incubator at 37°C for at least 15 min before transfer. The inner embryo transfer catheter, which carried the embryo-containing medium column, was maintained at room temperature until embryo loading.

Group C, combined inner and outer catheter incubation: both the sterile inner catheter and the outer sheath were placed in a CO2 incubator at 37°C for at least 15 min before embryo loading and transfer.

ART cycle preparation and embryo assessment

Fresh ET cycles followed individualized ovarian stimulation according to patient age, ovarian reserve, body mass index, and previous ovarian response. Final oocyte maturation, oocyte retrieval, fertilization by conventional IVF or ICSI, and luteal support were performed according to centre protocol. Frozen-thawed ET cycles followed either hormone replacement or ovulatory-cycle preparation according to clinical indication.

Embryos were assessed using standard morphological criteria. Cleavage-stage embryos were evaluated by cell number, blastomere symmetry, multinucleation, and fragmentation. Blastocysts were graded by expansion, inner cell mass quality, and trophectoderm appearance according to standard blastocyst grading principles [5, 9].

Embryo loading and transfer procedure

All transfers were performed using the same model of sterile soft ET catheter used routinely at the centre to maintain procedural uniformity. Embryo loading was carried out by an experienced embryologist under a laminar airflow hood. The loading sequence followed standard laboratory practice: medium column-air bubble-embryo-containing medium-air bubble-medium column. Loading time was standardized to 60 seconds or less for all groups.

Embryo transfer was performed by experienced clinicians under transabdominal ultrasound guidance. The catheter was advanced gently into the uterine cavity, and embryos were deposited approximately 1.5 cm from the uterine fundus. After transfer, the catheter was immediately examined under a stereomicroscope to confirm absence of retained embryos. Difficult transfer, blood or mucus contamination, and retained embryo events were documented.

Temperature and quality-control measurements

Incubator temperature, room temperature, and transfer-area environmental conditions were recorded as part of the laboratory quality-control program. In a predefined quality-control subset, catheter tip or loading-column temperature was measured with a calibrated thermocouple probe during simulated or non-embryo handling runs to avoid direct embryo exposure to measurement equipment. Temperature measurements were used to characterize group-level thermal profiles and were not used to alter clinical care or group classification.

Because these temperature measurements were collected for quality-control characterization rather than as participant-level clinical measurements, they were interpreted as supportive procedural data.

Outcome measures

Primary outcomes were clinical pregnancy rate and live birth rate per ET cycle. Clinical pregnancy was defined as an intrauterine gestational sac with fetal cardiac activity on ultrasound. Live birth was defined as delivery of a live infant after 24 completed weeks of gestation.

Secondary outcomes included biochemical pregnancy, ongoing pregnancy beyond 12 weeks, implantation rate, miscarriage rate, difficult transfer rate, retained embryo rate, and catheter temperature profile. Biochemical pregnancy was defined as a positive serum beta-human chorionic gonadotropin test 12–14 days after ET. Implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred. Miscarriage was defined as pregnancy loss before fetal viability.

Study size and statistical analysis

Because this was a retrospective analysis, no prospective sample size calculation was used to determine the study size. All eligible cycles with complete outcome follow-up within the data-extraction frame were included, yielding 80 cycles in each catheter handling group.

Analyses were performed according to the catheter handling strategy recorded in the clinical and embryology records. Continuous variables were assessed for normality using the Shapiro-Wilk test and inspection of histograms. Normally distributed variables were summarized as mean +/- standard deviation (SD), and non-normal variables were summarized as median and interquartile range (IQR). Categorical variables were summarized as number and percentage.

Comparisons among the three groups used analysis of variance or Kruskal-Wallis tests for continuous variables and Chi-square or Fisher exact tests for categorical variables, as appropriate. Pairwise comparisons were performed only when the overall test was significant and were interpreted as exploratory because no formal multiplicity correction was applied. Implantation rate was compared at the embryo level and interpreted cautiously because embryos were clustered within transfer cycles.

Multivariable logistic regression was used to estimate adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for live birth after adjustment for maternal age, embryo stage, transfer type, endometrial thickness, number of embryos transferred, and transfer of at least one top-quality embryo. A two-sided P value less than 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

Results

Participant flow and baseline characteristics

A total of 286 ET cycles performed between January 2024 and July 2025 were screened for eligibility. Forty-six cycles were excluded: 31 did not meet eligibility criteria and 15 had incomplete outcome follow-up. The final analysis included 240 cycles, with 80 cycles in Group A, 80 in Group B, and 80 in Group C.

Baseline demographic, clinical, and embryological characteristics were comparable across the three groups (Table 1). There were no statistically significant differences in maternal age, body mass index, ovarian reserve, endometrial thickness, fresh versus frozen-thawed ET, cleavage-stage versus blastocyst-stage transfer, number of embryos transferred, or transfer of at least one top-quality embryo.

Table 1
Baseline characteristics

Characteristic	Group A Inner only (n = 80)	Group B Outer only (n = 80)	Group C Inner + outer (n = 80)	P value
Maternal age, years, median (IQR)	33.7 (30.5–36.9)	34.0 (31.0-37.5)	33.5 (30.8–36.7)	0.82
Body mass index, kg/m2, mean +/- SD	25.0 +/- 3.8	25.2 +/- 4.0	24.8 +/- 3.7	0.79
Primary infertility, n (%)	45 (56.3)	43 (53.8)	44 (55.0)	0.95
AMH, ng/mL, median (IQR)	2.1 (1.1–3.6)	2.0 (1.0-3.5)	2.2 (1.1–3.7)	0.75
Endometrial thickness, mm, mean +/- SD	9.8 +/- 1.4	9.7 +/- 1.5	9.9 +/- 1.3	0.68
Fresh embryo transfer, n (%)	38 (47.5)	40 (50.0)	39 (48.8)	0.95
Blastocyst-stage transfer, n (%)	44 (55.0)	43 (53.8)	45 (56.3)	0.95
No. of embryos transferred, median (IQR)	1 (1–2)	1 (1–2)	1 (1–2)	0.88
At least one top-quality embryo transferred, n (%)	51 (63.8)	50 (62.5)	52 (65.0)	0.94

Catheter temperature profile and procedural variables

Quality-control temperature monitoring showed measurable thermal differences between catheter handling strategies (Table 2). The outer sheath-only group had the lowest inner catheter/loading-column temperature at loading because the embryo-carrying inner catheter was not incubated. The combined incubation group showed the most stable temperature profile from loading to the simulated 60-s time point. Median embryo loading time and laboratory-to-transfer transit time were similar across groups.

Difficult transfer and retained embryo events were uncommon and did not differ significantly among groups.

Table 2
Catheter temperature and handling variables

Variable	Group A Inner only	Group B Outer only	Group C Inner + outer	P value
Incubator temperature, °C, mean +/- SD	37.0 +/- 0.2	37.0 +/- 0.2	37.0 +/- 0.2	0.99
Room temperature, °C, mean +/- SD	25.1 +/- 0.8	25.0 +/- 0.9	25.1 +/- 0.8	0.91
Inner catheter/loading-column temperature at loading, °C, mean +/- SD	36.2 +/- 0.5	34.1 +/- 0.7	36.5 +/- 0.4	< 0.001
Estimated temperature at 60 s after loading, °C, mean +/- SD	35.8 +/- 0.6	33.6 +/- 0.8	36.2 +/- 0.5	< 0.001
Embryo loading time, s, median (IQR)	42 (35–50)	43 (36–51)	41 (34–49)	0.63
Laboratory-to-transfer transit time, s, median (IQR)	58 (47–70)	59 (48–72)	57 (46–69)	0.72
Difficult transfer, n (%)	4 (5.0)	5 (6.3)	4 (5.0)	0.91
Retained embryo, n (%)	1 (1.3)	1 (1.3)	0 (0.0)	0.60

Temperature values were obtained during simulated or non-embryo quality-control runs. IQR: interquartile range; SD: standard deviation.

Pregnancy and live birth outcomes

Pregnancy outcomes differed significantly across catheter handling groups (Table 3). Clinical pregnancy occurred in 42 cycles (52.5%) in Group A, 28 cycles (35.0%) in Group B, and 47 cycles (58.8%) in Group C (P = 0.008). Live birth occurred in 35 cycles (43.8%) in Group A, 22 cycles (27.5%) in Group B, and 40 cycles (50.0%) in Group C (P = 0.011).

Compared with outer sheath-only incubation, live birth was higher with inner catheter incubation alone (odds ratio 2.05, 95% CI 1.06–3.97, P = 0.047) and with combined inner plus outer incubation (odds ratio 2.64, 95% CI 1.37–5.09, P = 0.006). The absolute live-birth difference compared with Group B was 16.3 percentage points for Group A and 22.5 percentage points for Group C. Combined incubation showed numerically higher live birth than inner catheter incubation alone, but this difference was not statistically significant.

Table 3
Pregnancy and live birth outcomes

Outcome	Group A Inner only (n = 80)	Group B Outer only (n = 80)	Group C Inner + outer (n = 80)	P value
Biochemical pregnancy, n (%)	49 (61.3)	35 (43.8)	53 (66.3)	0.010
Clinical pregnancy, n (%)	42 (52.5)	28 (35.0)	47 (58.8)	0.008
Ongoing pregnancy > 12 weeks, n (%)	37 (46.3)	24 (30.0)	42 (52.5)	0.012
Live birth, n (%)	35 (43.8)	22 (27.5)	40 (50.0)	0.011
Gestational sacs/embryos transferred, n/N (%)	50/112 (44.6)	31/111 (27.9)	56/113 (49.6)	0.003
Miscarriage per clinical pregnancy, n/N (%)	5/42 (11.9)	4/28 (14.3)	5/47 (10.6)	0.90

Outcome categories are not mutually exclusive. Clinical pregnancy, ongoing pregnancy, and live birth are downstream outcomes after biochemical pregnancy.

Multivariable analysis

In multivariable logistic regression adjusted for maternal age, embryo stage, fresh versus frozen-thawed transfer, endometrial thickness, number of embryos transferred, and transfer of at least one top-quality embryo, catheter handling remained associated with live birth (Table 4). Using outer sheath-only incubation as the reference group, inner catheter incubation and combined incubation were both associated with higher odds of live birth.

Table 4
Logistic regression for live birth

Variable	Adjusted OR	95% CI	P value	Interpretation
Group A: inner catheter incubation vs Group B	1.97	1.02–3.82	0.043	Higher live birth odds
Group C: inner + outer incubation vs Group B	2.56	1.31–4.98	0.006	Higher live birth odds
Blastocyst-stage transfer	1.48	1.01–2.18	0.047	Favorable association
Endometrial thickness, per 1 mm	1.16	1.02–1.32	0.022	Favorable association
Maternal age, per year	0.96	0.91–1.02	0.18	Not significant after adjustment
Fresh transfer vs frozen-thawed transfer	0.92	0.55–1.55	0.75	Not significant

Group B served as the reference group for catheter handling.

Discussion

In this retrospective comparative cohort of 240 ET cycles, catheter handling strategy was associated with pregnancy and live birth outcomes. Inner catheter incubation and combined inner plus outer catheter incubation were associated with higher clinical pregnancy, ongoing pregnancy, and live birth rates than outer sheath-only incubation. Combined incubation had the numerically highest outcomes, although the difference between combined incubation and inner catheter incubation alone was not statistically significant.

The finding is biologically plausible because the inner catheter directly contains the embryo-loaded medium. Warming the outer sheath may improve the temperature of the guiding component, but it does not necessarily warm the lumen that contacts the embryo-containing medium before loading. By contrast, pre-incubating the inner catheter reduces the temperature gradient at the embryo-medium-catheter interface. Combined incubation may add thermal buffering during catheter assembly and passage through the sheath, which may explain the numerically higher outcomes observed in Group C.

Current ET guidance emphasizes soft catheter use, ultrasound guidance, atraumatic technique, and standardization of the transfer procedure [1]. ART laboratory guidance similarly emphasizes environmental control, traceability, quality management, and minimization of non-physiological exposure during gamete and embryo handling [2, 3]. However, these documents do not specify whether the inner catheter, outer sheath, or both components should be pre-incubated. The present study addresses this practical laboratory-clinical question using live birth as a clinically meaningful outcome.

The temperature findings support the clinical observations but should be interpreted carefully. The lowest loading-column temperature occurred when only the outer sheath was incubated, which is consistent with the fact that the embryo-carrying inner catheter remained at room temperature. Previous simulated transfer studies have shown that catheter contents can cool rapidly during the interval between loading and uterine placement [7, 8]. In the present study, temperature monitoring was performed in simulated or non-embryo quality-control runs to avoid exposing embryos to measurement devices; therefore, the temperature data characterize the handling protocols rather than providing direct embryo-level temperature measurements.

The study has several strengths. It used a defined transfer period, comparable group sizes, uniform eligibility criteria, standardized ultrasound-guided transfer technique, a restricted loading time, and live birth as a primary outcome. Baseline demographic, clinical, and embryological variables were similar across groups, and the association between catheter handling and live birth persisted after adjustment for major clinical and embryological factors.

The study also has limitations. Its retrospective design means that unmeasured confounding and temporal changes in workflow cannot be excluded. Temperature measurements were obtained from simulated or quality-control runs rather than embryo-containing transfers. Implantation rate was calculated per embryo transferred, but embryos were clustered within transfer cycles; therefore, implantation results should be interpreted as exploratory. The findings may also vary according to catheter model, laboratory-to-procedure-room distance, ambient temperature, operator workflow, and embryo transfer timing.

Catheter incubation is simple and inexpensive, but routine adoption should be based on reproducible evidence. These data suggest that warming the embryo-carrying inner catheter may be more relevant than warming the outer sheath alone. Prospective multicentre studies, preferably with standardized catheter temperature monitoring and predefined adjustment for embryo and uterine factors, are needed to confirm whether catheter incubation strategy independently improves clinical outcomes.

Conclusions

In this multi-centre retrospective cohort study, inner catheter incubation and combined inner plus outer catheter incubation were associated with higher clinical pregnancy and live birth rates than outer sheath-only incubation. Combined incubation produced the most favorable numerical outcomes. These findings suggest that thermal stability of the embryo-carrying inner catheter may be clinically relevant during ET. Larger prospective studies with standardized temperature monitoring are needed before incorporation into routine embryo transfer standard operating procedures.

Abbreviations

AMH
anti-Mullerian hormone

aOR
adjusted odds ratio
ART
assisted reproductive technology
CI
confidence interval
CO2
carbon dioxide
ET
embryo transfer
ICSI
intracytoplasmic sperm injection
IQR
interquartile range
IVF
in vitro fertilization
OR
odds ratio
SD
standard deviation
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee of Directorate of Health Services, Goa [approval number: DHS/Sp-Cell/24–166(Ethics)/2025-26/1208, dated 19/08/2025]. The study used de-identified routine clinical records, and the requirement for individual written consent for this retrospective analysis was waived by the approving committee. The study was conducted in accordance with the Declaration of Helsinki and applicable Indian ART regulations.

Consent for publication

Not applicable. The manuscript does not contain individual participant details, images, or videos.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

CVS conceptualized the study, supervised the laboratory protocol, interpreted embryology procedures, and critically revised the manuscript. SKSP contributed to study coordination, data collection, statistical analysis, and drafting of the manuscript. AAN contributed to clinical embryo transfer procedures, clinical data interpretation, and manuscript revision. AJK contributed to clinical oversight, interpretation of reproductive outcomes, and final manuscript refinement. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Data Availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request, subject to institutional ethics approval and applicable confidentiality requirements for ART data.

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