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Assisted Hatching and Intracytoplasmic Sperm Injection are not Associated with Improved Outcomes in ART Cycles for Diminished Ovarian Reserve: An Analysis of US Cycles from 2004–2011

Samantha F. Butts, MD MSCE¹, Carter Owen, MD¹, Monica Mainigi, MD¹, Suneeta Senapati, MD MSCE¹, David B. Seifer, MD^{2,3}, and Anuja Dokras, MD PhD¹

¹Department of Obstetrics and Gynecology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

²Genesis Fertility and Reproductive Medicine, Maimonides Medical Center, Brooklyn, New York

³New York University School of Medicine, New York City, New York

Abstract

Objective—To investigate the impact of intracytoplasmic sperm injection (ICSI) and assisted hatching (AH) on ART outcomes in cycles with diminished ovarian reserve (DOR) as the primary diagnosis.

Design—Retrospective cohort study of cycles from the SART-CORS database.

Setting—NA.

Patient(s)—A total of 422,949 fresh, non-donor, initial ART cycles of which 8,597 were diagnosed with only elevated FSH and 38,926 were diagnosed with only DOR according to the SART DOR categorization.

Intervention(s)—None.

Main Outcome Measure(s)—Live birth and clinical pregnancy rates.

Result(s)—ICSI and AH were associated with diminished odds of live birth in SART DOR only cycles (AOR, 95% CI 0.88, 0.81–0.96 for ICSI; AOR, 95% CI 0.77 0.71–0.84 for AH). No association between either ICSI or AH in Elevated FSH only cycles was observed. The

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Corresponding Author: Samantha F. Butts, MD MSCE, Assistant Professor of Obstetrics and Gynecology, Division of Infertility and Reproductive Endocrinology, Perelman School of Medicine, University of Pennsylvania, 3701 Market Street, Suite 800, Philadelphia Pennsylvania 19104, 215-662-2975 (p), 215-349-5512 (f), sbutts@obgyn.upenn.edu.

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combination of ICSI and AH resulted in significantly lower odds of live birth in SART DOR only cycles but not in Elevated FSH only cycles.

Conclusion(s)—In initial ART cycles for which the only indication relates to a diagnosis of diminished ovarian reserve, assisted hatching and ICSI are not associated with improved live birth rates.

Keywords

Diminished ovarian reserve; ICSI; assisted hatching; ART

INTRODUCTION

Of all the causes of infertility treated with assisted reproductive technologies (ART) diminished ovarian reserve (DOR) persistently lags behind alternate diagnoses in the proportion of cycles that achieve a live birth (1). DOR has traditionally been characterized as an infertility-associated state of limited response to exogenous gonadotropins in which the quality of oocytes may also be diminished (2–10). Data from the SART/CDC registry of national ART cycles has regularly demonstrated that live birth rates in women with DOR are 40–50% lower than observed with any other diagnosis for which ART is sought (1). These data have led many in the field to adopt the practice of using adjunct therapies in ART such as micromanipulation of gametes and embryos with the intent to maximize the chances of successful pregnancy outcome.

Micromanipulation techniques including assisted hatching (AH) and intracytoplasmic sperm injection (ICSI) were first introduced to optimize outcomes for specific indications in IVF (11, 12). Hatching of the embryo through the zona pelucida is a necessary step for successful implantation and it has been suggested (13–15) that barriers to hatching may limit pregnancy rates in assisted reproduction. Increasing maternal age, elevated FSH concentrations, and even embryo culture have been proposed as risk factors for zona pelucida thickening and/or hardening that could challenge embryo hatching and implantation (11, 14, 16, 17). More than 20 years ago, the first clinical reports were published supporting the incorporation of AH into IVF as a method that could improve the ability of certain embryos to implant (13, 15, 18). Since the publication of those initial reports, multiple approaches to thinning and breaching the zona pelucida have been proposed (11, 17). However, the data supporting the benefit of AH in ART cycles has been variable and contradictory (11, 19–25). Much of the data suggesting a benefit of AH has found improvements in cycles following prior IVF failures, cycles treating women at least 38 years of age, or cycles in which embryo quality is poor (11, 14, 26). A role for AH in improving ART outcomes for women with DOR has not been demonstrated to date.

ICSI was first introduced as a means of improving fertilization in the presence of severe male factor infertility or prior IVF with fertilization failure (12, 27). Over time, the indications for ICSI have broadened leading to its current incorporation into many ART cycles without male factor infertility. According to recent data from the Society for Assisted Reproductive Technology (SART), IVF with ICSI comprised more than 70% of all procedures performed in 2010 and more than half of the ICSI cycles performed applied to

cycles with no male factor infertility (28). While there is often a concern for poor fertilization in DOR cycles based on limited numbers of oocytes and the presumption of poor oocyte quality, it has been noted that the use of ICSI in cycles where there is no male factor does not routinely improve ART outcomes (12, 29, 30). Even when IVF cycles in poor responders have been investigated, ICSI was not associated with increases in either fertilization or implantation rates compared to conventional IVF (31). The only report to date that has evaluated the incorporation of ICSI in low responders with elevated FSH did not find an improvement in pregnancy compared to fertilization with conventional IVF (31).

In recommending adjuncts to ART, the benefit of such interventions must be weighed against potential clinical risks and financial costs. Several reports have linked AH and ICSI to pregnancy complications such as monozygotic twinning, sex chromosome aneuploidy, and fetal anomalies (32–35). Recognizing the need for evidence in this area to guide treatments, the primary aim of this investigation was to determine the association between micromanipulation and IVF treatment outcomes (clinical pregnancy) and pregnancy outcomes (live birth) in fresh IVF cycles treating couples whose only diagnosis is DOR. We hypothesized that in ART for diminished ovarian reserve, cycles utilizing assisted hatching and/or ICSI have similar treatment outcomes to cycles that do not utilize these techniques. To test this hypothesis, cycles from the national registry of ART cycles and retrieved from Society for Assisted Reproductive Technologies-Clinical Outcome Reporting System (SART-CORS) were analyzed for study.

MATERIALS AND METHODS

Data Source and Outcome Measures

This study was reviewed by the Office of Regulatory Affairs at the University of Pennsylvania Medical Center and allowed an exemption from institutional review board approval. The data source for the study was the SART-CORS database, a registry that contains comprehensive data regarding ART cycles submitted by US clinics to the Society for Reproductive Medicine (SART) and reported to the Centers for Disease Control and Prevention in compliance with the Fertility Clinic Success Rate and Certification Act of 1992. Greater than 90% of all clinics providing ART in the United States are compliant with the mandate to report (28, 34, 42). The data set for this investigation included de-identified fresh non-donor ART cycles performed between 2004 and 2011. Cycles performed for the purposes of banking of oocytes or embryos are not included in the data set (28).

Two approaches were used to identify cycles as DOR in the dataset. The first approach was to consider any cycle in which the *Reason for ART* field listed DOR as a case of DOR. For any given treatment cycle, there may be a solitary reason for ART or multiple diagnoses linked to a given cycle. Diminished ovarian reserve, for instance, can be the sole reason for a couple pursuing ART (DOR only), or it can be combined with other female and/or male infertility factors. For the purpose of testing associations between micromanipulation and treatment outcomes in DOR cycles (here to for labeled as SART DOR category) cycles identified with multiple diagnoses were excluded.

Based on the assumption of heterogeneity in the diagnosis of DOR collected from multiple clinics, a second category for DOR was derived. This category focused on FSH elevation as the primary indicator of DOR. Early follicular phase FSH elevation was derived from the *Patient Maximum FSH Level SART field* along with an assessment of the distribution of FSH values in the sample. The range that was characterized as abnormal represented the 90%-99% of values (extreme outliers were excluded) and corresponded to FSH values of 12–24 IU/L. Elevated FSH cycles were then further distinguished as those excluding additional infertility diagnoses (elevated FSH only). Because many clinics define elevated FSH as a concentration of 10 IU/L or greater, a second category using a range of 10–24 IU/L was devised for testing associations between micromanipulation and live birth.

The data analysis further focused on cycles (the unit of analysis) among women with no prior ART to eliminate repeated cycle bias. Women up to age 44 were included in the analysis. Cycles utilizing rescue ICSI were excluded. In univariate analyses, ICSI (captured in the dataset as performed to all or some oocytes) and AH (to all or some embryos) were characterized as dichotomous variables. In multivariable regression models for live birth, the association between live birth and ICSI alone, AH alone and both in combination was determined.

The primary ART outcome measured was live birth per cycle initiated. Additional outcomes included, fertilization rate, clinical pregnancy rate, and probability of monozygotic multiple gestation.

Statistical Methods

Associations between categorical variables were tested using the χ^2 test. Comparisons of continuous variables between groups were made using the Wilcoxon rank sum and Kruskal-Wallis tests, as appropriate. Relative risks were used to characterize univariate tests of association between micromanipulation and live birth. Logistic regression was used to model the impact of AH alone, ICSI alone or AH combined with ICSI on odds of live birth in cycles with SART DOR category or Elevated FSH as a sole infertility diagnosis. Because of the large number of observations and comparisons in the data set, the stringency of statistical tests was adjusted to account for possible significant associations with limited clinical significance. Statistical tests were therefore set at significance level of $p < 0.0001$. Tests with $0.0001 \leq p < 0.05$ were deemed borderline significant.

All statistical tests were performed using STATA 12 software (StataCorp).

RESULTS

A total of 422,949 fresh, non-donor, first ART cycles were identified of which 38,926 were associated with DOR as the only diagnosis (SART DOR category only) and of which 8,597 were associated with elevated FSH as the sole diagnosis (Elevated FSH only). ICSI was performed less often in cycles with SART DOR category only (61.2%) or Elevated FSH only (59.3%) than in Normal FSH (70%) or Non- SART DOR category cycles (control cycles (69.3%, $p < 0.0001$ for all comparisons) (Supplemental Table 1). Conversely, AH was more often performed in SART DOR category only (52.9%) and Elevated FSH only

(55.9%) cycles than in Normal FSH (35%) and Non- SART DOR category cycles (33.5%, $p<0.0001$ for all comparisons). In nearly one-third of all initial ART cycles, regardless of diagnosis, both AH and ICSI were performed (32.9%). The proportion of SART DOR category only cycles in which both AH and ICSI were performed was significantly higher than in Non- SART DOR category cycles (34.9% and 25.9% respectively, $p<0.0001$). Likewise, a greater proportion of Elevated FSH only cycles than Normal FSH cycles had both ICSI and AH performed (34.1% vs. 26.8%, $p<0.0001$).

In Elevated FSH only cycles, higher rates of fertilization proportion of blastocysts transferred and good quality embryos were observed in cycles that incorporated ICSI than in those that did not (Table 1). Conversely, clinical pregnancy rates were diminished when ICSI was used in Elevated FSH only cycles. When AH was performed in these cycles, all treatment outcomes, including clinical pregnancy rate were more favorable than when AH was not used (Table 1). Neither ICSI nor AH was associated with monozygotic multiple gestations in Elevated FSH only cycles.

In SART DOR category only cycles incorporating ICSI compared to those that did not, fertilization rates were similar, embryo quality (quality for up to three embryos listed) was more favorable, and clinical pregnancy rates were significantly diminished (Table 2). SART DOR category only cycles in which AH was utilized experienced significantly lower clinical pregnancy rates than those in which it was not performed (27.4% for AH, 28.7% for no AH, $p<0.0001$). Neither ICSI nor AH was associated with monozygotic multiple gestations in DOR only cycles.

Additional univariate analyses were performed focusing on live birth as the outcome (Table 3). No significant association between live birth and micromanipulation were observed in Elevated FSH only cycles. Both ICSI and AH were associated with diminished live birth rates in SART DOR category only cycles (20.4% in ICSI cycles vs. 21.9% in cycles without ICSI, RR of live birth 0.93, $p=0.002$; 19.5% in AH cycles vs. 22.6% in cycles without AH RR of live birth 0.86, $p<0.0001$).

Multivariable logistic regression models were fit to test the association between micromanipulation (ICSI, AH, or both) and live birth rates in Elevated FSH only cycles and SART DOR category only while adjusting for multiple confounders (Table 4). No association was observed between either ICSI or AH and live birth in Elevated FSH only cycles (AOR 0.93 95% CI 0.77–1.1, $p=0.5$ for ICSI; AOR 0.99, 95% CI 0.8–1.2, $p=0.9$ for AH). Odds of live birth were not associated with the combination of ICSI and AH in Elevated FSH only cycles. When expanding the definition of Elevated FSH only to include cycles in which the range of FSH was 10–24 IU/L, there was no change in the null associations observed between micromanipulation procedures and odds of live birth.

In SART DOR category only cycles, ICSI was associated with 13% lower odds of live birth than no ICSI (AOR 0.87, 95% CI 0.79–0.95, $p=0.002$) while AH was associated with 16% lower odds of live birth than no AH (AOR 0.84 95% CI 0.77–0.92, $p<0.0001$). The combination of ICSI and AH was associated with a 27% lower odds of live birth compared

to SART DOR category only cycles in which neither was performed (AOR 0.73, 95% CI 0.65–0.82).

Because AH is often performed in ART cycles based on age, additional evaluation focused on whether age modified the effect of AH on live birth. The association between AH and live birth was not found to vary according to age in either DOR only cycles or Elevated FSH only cycles. Similarly, the association between ICSI and odds of live birth did not vary according to age (Table 4).

DISCUSSION

Given the poor prognosis for many DOR cycles, the notion that the yield and/or implantation of embryos from DOR cycles could be improved by the utilization of ICSI and AH respectively is very clinically appealing. Such practices however, are not supported by strong evidence. Therefore, the aim of this investigation was to determine the association between live birth and micromanipulation (AH and/or ICSI) in ART cycles performed when the specific indication was diminished ovarian reserve. Neither AH or ICSI improved the odds of live birth in initial ART cycles for the treatment of DOR and in some cycles these adjuncts we associated with a reduced likelihood of live birth.

Two main DOR exposure groups were evaluated in this investigation. Primarily, we chose to categorize cycles as having a diagnosis of elevated FSH if associated with a “Patient Maximum FSH” in the 90–99% of values (range of values 12–24 IU/L, 8,597 first ART cycles). This categorization provided a unified, biomarker-based definition of DOR. In addition, cycles were classified as falling into the SART DOR category according to the diagnosis compiled from reporting clinics and then submitted to the data set (38,926 first ART cycles). The SART definition of DOR is “a reduced ability of the ovary to produce eggs due to either advanced age, congenital, medical, or surgical causes” (1) and leaves open the possibility of significant heterogeneity in patients characterized as DOR in SART-CORS. Cycles fulfilling either of these criteria were then isolated from other causes of infertility so as to assess DOR separate from other diagnoses. When focusing on Elevated FSH cycles alone, neither AH or ICSI made a significant impact on clinical pregnancy rates or the adjusted odds of live birth. This remained the case when extending the range of Elevated FSH to include values between 10–24 IU/L. These data stand in contrast to the observation that embryo quality was superior in cycles in which ICSI was performed. While the difference in proportions of good quality embryos favoring ICSI cycles was statistically significant, the absolute difference between groups was small. These small differences may not have been sufficient to significantly impact ultimate pregnancy outcomes in a poor prognosis group of subjects.

The overall impact of AH and ICSI on cycles characterized as SART DOR category only was less favorable than in cycles with elevated FSH. The reason for the variation in the association between micromanipulation and ART outcomes in the two DOR categories investigated is not readily apparent. As was noted, the DOR category compiled in SART-CORS relies on the interpretation of many different clinics and this diagnosis is likely to vary from clinic to clinic. It is possible that micromanipulation impacts ART outcomes for

DOR cycles diagnosed in different ways, according to distinct validated markers of ovarian reserve. While our adjusted analysis controlled for embryo quality, it remains possible that gametes and embryos derived from some DOR cycles could be more vulnerable to the potential risks of micromanipulation than those derived from Elevated FSH cycles.

The results of this investigation are notable given the large number of cycles permissible for study through SART-CORS and the limited literature that has assessed ICSI and AH in DOR cycles exclusively. While there have been multiple reports comparing ART outcomes in cycles with and without AH, most have defined poor prognosis as prior failed cycles or based on maternal age rather than looking specifically at FSH concentration. Many such studies support a role for AH in improving clinical pregnancy rates in women who are at least 38 years old or who have had unsuccessful prior ART cycles; several studies have also demonstrated improvements in ongoing/live birth rates in these subpopulations (19–22, 36). In contrast, Assemi et al published a study more analogous to ours in its comparison of 154 initial ART cycles all with FSH ≥ 10 IU/L according to whether AH was performed. Similar to the results reported herein, Assemi et al. observed that clinical pregnancy rates were lower in subjects who received AH than in those who did not (43.6% and 56.8% respectively); however, this difference was not statistically significant ($p=0.14$) and live birth rates were not reported.

In contrast to several other published reports, we did not find that maternal age modified the association between AH and live birth rates in either SART DOR category only or Elevated FSH only cycles. It is possible that in an unselected population of chronologically older women without a diagnosis of DOR, that AH is more beneficial than in our specific sample. We also did not find that micromanipulation increased the risk of monozygotic multiple gestations in women with SART DOR category or Elevated FSH. This phenomenon has been described in several other populations (33, 34, 37) but may not apply to the specific diagnoses study herein.

While ours is not the only report that has tested the role of ICSI in non-male-factor ART cycles, it is first that has evaluated the association between ICSI and live birth in DOR only cycles. In general, the utilization of ICSI in cycles without male factor has not improved treatment outcomes in ART cycles (12, 29, 31, 38) and has not reliably prevented fertilization failure compared to conventional IVF. An exception may be the case of unexplained infertility cycles; several recent reports have suggested an improvement ART outcomes when ICSI is used (39–41). A search of the literature identified two publications --- one a randomized trial -- specifically addressing the impact of ICSI on ART outcomes in cycles characterized by low response to gonadotropins (less than 6 and less than 4 oocytes retrieved) (29, 31). There was no improvement in fertilization rate or clinical pregnancy rate when ICSI was applied to oocytes from patients with poor response. In the study that reported on ovarian reserve testing, FSH values in the conventional IVF and ICSI groups were elevated (12.4 IU/L and 12.1 IU/L respectively) and comparable across treatment groups (31). Our findings build on the results of these previous reports by studying a much larger sample size and by focusing on live birth as a primary outcome.

Studying thousands of ART cycles provided sufficient power to test inferences that would be challenged in a smaller sample. A trade off for this increased power is that very subtle differences of uncertain clinical significance can be associated with p values less than 0.05. To address this problem we adjusted our threshold for statistical significance to $p < 0.0001$ and carefully evaluated all comparisons emphasizing those that were both statistically and clinically significant. By design, our investigation focused on the first cycles of ART in women with DOR as a sole infertility diagnosis. This approach was utilized to isolate the impact of AH and ICSI on DOR cycles in which there was no apparent reason -- such as male factor infertility, prior ART with failed fertilization, or repeated implantation failure -- to incorporate these treatments. Focusing on initial cycles of ART also limited the selection bias that can occur when evaluating repeated IVF cycles in individual patients (42).

Despite the strengths of this study some limitations exist. We do not have information on the methods used to perform AH in the various cycles studied and hence cannot determine the association between specific AH procedures and outcomes. While our assessment of Elevated FSH only cycles allowed for an evaluation of cycles using a strict biochemical definition of DOR, we cannot define the impact of micromanipulation on women with low AMH but normal FSH as there is still limited accrual of AMH data in SART-CORS. The women defined as DOR by SART could encompass alternate definitions of DOR such as low AMH, low antral follicle count, or poor response in a prior COH cycle. Since we do not have these data for each cycle we are unable to test associations between micromanipulation and the outcome of cycles of women who fall into these specific DOR categories. It should be noted that by design, our results apply only to women receiving an initial cycle of ART without infertility diagnoses beyond DOR; our results may not be generalizable to women with multiple infertility diagnoses or those pursuing multiple ART cycles.

Ultimately, the most internally valid approach to investigating the impact of micromanipulation on DOR cycles would be a randomized controlled trial to minimize the selection bias and uncontrolled confounding that can occur in observational studies such as ours. Since such a trial has not occurred to date, we believe that this investigation is a well designed observational approach to testing the association between micromanipulation and ART outcomes in a select group of patients with DOR -- those cycling for the first time without an additional infertility diagnosis.

CONCLUSIONS

In the Elevated FSH and DOR cycles studied for this investigation, greater than 50% utilized ICSI or assisted hatching. The decision to integrate any treatment into an ART cycle should be made with a thorough understanding of its associated benefits, risks, and costs. While this investigation used large numbers of cycles to test the impact of micromanipulation on the outcome of ART in DOR cycles, it is also important for individual clinics to critically scrutinize their own practice utilization and outcomes. Nevertheless, based on this assessment of national data over 8 years, we conclude that the empiric use of AH and/or ICSI in initial ART cycles does not improve treatment outcomes for women whose only diagnosis is DOR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

ART Treatment Outcomes in Elevated FSH Only According to use of Micromanipulation

	ICSI (n=3686 Cycles)	No ICSI (n=2527 Cycles)	P value	AH (n=3475 Cycles)	No AH (n=2742 Cycles)	P value
Fertilization Rate Mean $\pm$ SD	50.9% $\pm$ 34.4	46.4% $\pm$ 35.8	0.0003			
% with Blastocyst Tx	10.3% (381)	7.9% (200)	0.001	3.9%	16.2%	<0.0001
% Good Quality Embryos #1 *	32.6% (1203)	25.7% (650)	<0.0001			
% Good Quality Embryos #2 *	20.9% (771)	18.1% (457)	<0.0001			
% Good Quality Embryos #3 *	8.1% (297)	7.3% (185)	<0.0001			
# Embryos transferred	2.3 $\pm$ 1.08	2.4 $\pm$ 1.08	<0.0001	2.4 $\pm$ 1.2	2.1 $\pm$ 0.9	<0.0001
Clinical Pregnancy Rate	26.9% (990)	27.7% (699)	0.001	28.9% (1004)	25% (685)	<0.0001
% Monozygotic Multiple Gestations	0.16% (6)	0.2% (5)	0.7	0.2%	0.15%	0.6

* SART embryo morphology field

ICSI status not available for 2384 cycles, AH status not available for 2380 cycles

Table 2
ART Treatment Outcomes in SART DOR Only Category According to Use of Micromanipulation

	ICSI (n=18559 Cycles)	No ICSI (n=11789 Cycles)**	P value	AH (n=16057 Cycles)	No AH (n=14302 Cycles)**	P value
Fertilization Rate (Mean ±SD)	48.8%±34	48.1% ±34.7	0.8	----	----	----
% with Blastocyst Tx	14% (2598)	10.5% (1232)	<0.0001	5.7% (913)	20.4% (2919)	<0.0001
% Good Quality First Embryos Transferred*	33.6% (3319)	28.2% (6233)	<0.0001	----	----	----
% Good Quality Second Embryos Transferred*	24.1% (4474)	20.9% (2458)	<0.0001	----	----	----
% Good Quality Third Embryos Transferred*	10.6% (1959)	9.9% (1170)	<0.0001	----	----	----
# Embryos transferred Mean ±SD	2.6±1.2	2.7±1.2	<0.0001	2.7 ±1.3	2.4 ±1	<0.0001
Clinical Pregnancy Rate	27.4% (5075)	29.1% (3434)	<0.0001	27.4% (4405)	28.7% (4107)	<0.0001
% Monozygotic Multiple Gestations	0.18% (34)	0.21% (25)	0.6	0.15% (24)	0.24% (35)	0.06

* SART embryo morphology field

** ICSI status not available for 8578 cycles, AH status not available for 8,567 cycles

Table 3
Univariate Association of Micromanipulation and Live Birth in SART DOR Only Category and Elevated FSH Only Cycles

Risk Factor	% Elevated FSH Only Cycles resulting in Live Birth (n)	RR (95% CI) of LB in Elevated FSH Only Cycles -- Micromanipulation vs None	P	% SART DOR Only Cycles resulting in Live Birth (n)	RR (95% CI) of LB in SART DOR Only Cycles -- Micromanipulation vs None	P
No ICSI	22% (552/2514)	Ref	0.3	21.9% (2567/11,737)	Ref	0.002
ICSI	20.9% (761/3635)	0.95 (0.87–1.07)		20.4% (3744/18391)	0.93 (0.89–0.97)	
No AH	21% (562/2682)	Ref	0.5	22.6% (3190/14106)	Ref	<0.0001
AH	21.6% (751/3470)	1.03 (0.94–1.14)		19.5% (3123/16033)	0.86 (0.82–0.9)	

Adjusted Association of Micromanipulation and Live Birth in SART DOR Only Category and Elevated FSH Only Cycles

Table 4

Elev FSH Only – Adjusted OR (95% CI) of LB**	P value	Elev FSH Only (10–24 mIU/mL) – Adjusted OR (95% CI) LB***	P value	Risk Factor	SART DOR Only Category – Adjusted OR (95% CI) LB*	P value
Ref	0.5	Ref	0.5	No ICSI	Ref	0.002
0.93 (0.77–1.1)		0.95 (0.82–1.1)		ICSI	0.87 (0.79–0.95)	
Ref	0.9	Ref	0.3	No AH	Ref	<0.0001
0.99 (0.8–1.2)		0.92 (0.7–0.94)		AH	0.84 (0.77–0.92)	
Ref	0.6	Ref	0.2	No ICSI/No AH	Ref	<0.0001
0.93 (0.7–1.2)		0.87 (0.71–1.08)		ICSI +AH	0.73 (0.65–0.82)	

Adjusted for categorical age (<35, 35–37, 38–40, 41–42, >=43), gravidity, oocytes retrieved, #embryos transferred, embryo quality, blastocyst transfer, treatment year, AH, ICSI

* No interaction between age and AH on LB, interaction p=0.9; No interaction between age and ICSI on LB, p=0.98

** No interaction between age and AH on LB, interaction p=0.9; No interaction between age and ICSI on LB, p=0.7

*** No interaction between age and AH on LB, interaction p=0.8; No interaction between age and ICSI on LB, p=0.2