

# Neonate female to male ratio after assisted reproduction following antagonist and agonist protocols

Dania Al-Jaroudi, MD, MHA<sup>a,\*</sup>, Gamar Salim, MD<sup>a</sup>, Saeed Baradwan, MBBS<sup>b</sup>

## Abstract

We retrospectively compared neonatal sex after antagonist- versus long-stimulation protocols followed by fresh in vitro fertilization (IVF) or fresh intracytoplasmic sperm injection (ICSI) with either protocol. We reviewed data for 762 IVF/ICSI cycles in 2015, including 23 IVF procedures. We summarized sex outcomes in the entire cohort, and for the additional subgroups: embryo transfer day and number of embryos transferred, and number of oocytes recovered and maternal age. Among 169 live births for all protocols combined, 50.9% of babies were male, and we saw no difference between the antagonist versus long-stimulation groups (52.3% vs 48.3% male babies, respectively;  $P = .740$ ). Our results also showed no significant difference in sex proportion when comparing IVF versus ICSI, although a higher proportion of babies were male with the antagonist-ICSI protocol. Differences between the additional subgroups were also neither clinically nor statistically significant.

**Abbreviations:** ART = assisted reproductive technology, E2 = oestradiol, FSH = follicle-stimulating hormone, GnRH = gonadotropin releasing hormone, Hcg = human chorionic gonadotrophin, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, SSR = secondary sex ratio.

**Keywords:** agonist, antagonist, iCSI, iVF, sex

## 1. Introduction

A preference by some parents for male or female babies is seen in the increased interest in prenatal sex determination that accompanied rapid developments in assisted reproductive technology (ART). However, preimplantation genetic screening and other tools allowing reliable early sex determination are neither ethically approved nor available universally.<sup>[1]</sup> ART clinicians are frequently asked if other treatments are available to increase the chances of having a child of the patient's preferred sex. Numerous studies have explored the possible effects of parental characteristics and specific components of ART regimens and procedures on the secondary sex ratio (SSR) of ART babies. With few exceptions, studies support an increase in male babies following blastocyst transfer compared with

cleavage-stage embryos, and following in vitro fertilization (IVF) compared with intracytoplasmic sperm injection (ICSI).<sup>[2,3]</sup> However, data from patients' specific sociodemographic environments can be more meaningful, when answering patient questions regarding ART and sex. Accordingly, we examined outcomes for ART procedures performed in 2015 at our tertiary referral center, the Reproductive Endocrine and Infertility Medicine Department at King Fahad Medical City, Riyadh, Saudi Arabia. We focused on sex outcomes from antagonist compared with long stimulation protocols, for which few reports are available.

## 2. Methods

Our institutional review board approved this retrospective review of medical records for patients attending the Reproductive Endocrine and Infertility Medicine Department of our institution in 2015. We analyzed data for 762 treatment cycles initiated following antagonist ( $n=545$ ) and long ( $n=217$ ) protocols. Briefly, all patients undergoing IVF/ICSI underwent follicle-stimulating hormone (FSH), luteinizing hormone, and oestradiol ( $E_2$ ) level measurements and a baseline ultrasound examination during their second or third menstrual period after initial examination.

The gonadotropin releasing hormone (GnRH) antagonist protocol comprised daily injections of either recombinant FSH (GONAL- $\alpha$ , Merck Serono, Darmstadt, Germany) or human menopausal gonadotropin (Merional, IBSA, Lugano, Switzerland). The starting dose was 150 IU for all patients <30 years of age and 225 to 300 IU for those >35 years. Daily subcutaneous injections of 0.25 mg GnRH antagonist (Cetrotide, Serono, Geneva, Switzerland) were started on day 5 or 6 of menses or when the leading follicle was 13 to 14 mm in diameter and then continued daily until human chorionic gonadotrophin (hCG) (Pregnyl, Merck, Kenilworth, NJ) injections were started when at least 3 leading follicles reached 17-mm mean diameter.

Editor: Yan Li.

This work was supported by the King Fahad Medical City Intramural Research Fund under Grant Number 17-045.

The study was funded by KFMC.

Data availability statement: Data is available.

The authors have no conflicts of interest to disclose.

<sup>a</sup>Reproductive Endocrine and Infertility Medicine Department, <sup>b</sup>Department of Obstetrics and Gynecology, King Fahad Medical City, Riyadh, Saudi Arabia.

\* Correspondence: Dania Al-Jaroudi, Reproductive Endocrine and Infertility Medicine Department, King Fahad Medical City, P.O. Box: 59046, Riyadh 11525, Saudi Arabia (e-mails: daljaroudi@kfmc.med.sa, dania@dohaim.com)

Copyright © 2018 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Medicine (2018) 97:38(e12310)

Received: 3 June 2018 / Accepted: 15 August 2018

<http://dx.doi.org/10.1097/MD.0000000000012310>

Patients in the agonist group received 3.75 mg depot leuprolide acetate (Lupron, Abbott Laboratories, Chicago, IL) intramuscularly during the luteal phase of the previous cycle, once on day 21 of the cycle. Each patient was examined 12 to 15 days after these treatments for the absence of ovarian follicles, endometrial thickness  $\leq 6$  mm on transvaginal ultrasound examination, and plasma  $E_2$  levels  $< 250$  mmol/L to verify desensitization. Once desensitization was verified, patients began treatment with r-FSH or HMG at varying doses, as described earlier. Doses were adjusted after 5 or 6 days of stimulation based on ovarian response, which was evaluated using  $E_2$  levels and ultrasound. We used a step-up or step-down protocol as indicated after ultrasound and  $E_2$  measurement, and when 3 follicles reached a mean diameter  $\geq 17$  mm, patients received 10,000 IU hCG by intramuscular injection. Transvaginal ultrasound was used to assess follicular growth on day 5 or 6, and was repeated on day 8 or 9 and on the day that hCG was injected.

Retrieved oocytes were injected with sperm using IVF if the semen analysis was normal. ICSI was performed for severe male factor infertility or in patients experiencing previously failed fertilization after IVF. We transferred 2 fresh embryos on days 2 to 5 after fertilization. Patients were prescribed a 400 mg vaginal progesterone pessary (Cyclogest, Actavis, Barnstaple, UK) twice daily for luteal support, which was continued if pregnancy was maintained for 10 to 12 weeks. Clinical pregnancy was confirmed with vaginal ultrasound once increasing beta-hCG levels were detected.

Summary data were prepared for demographic and outcome data. Continuous variables were categorized into ordinal and nominal groups and summarized using proportions. The  $\chi^2$  or Fisher exact test was used to explore associations between sex outcomes. We summarized sex outcomes in the entire cohort, and in modifiable (e.g., embryo transfer day and number of embryos

transferred) and nonmodifiable (e.g., number of oocytes recovered and maternal age) subgroups.

### 3. Results

Most patients were  $< 40$  years of age (Table 1). The cause of infertility was unexplained in 20% of patients, and 19% had  $\geq 2$  factors contributing to their infertility. Almost half of the women had severe male factor infertility (47%), and approximately one-fifth (21%) had polycystic ovarian syndrome.

In 690 cycles, oocyte aspirations were completed successfully, with oocyte recovery from 671 (97.2%) aspirations. The average number of oocytes recovered from successful aspiration procedures was  $9.9 \pm 7.0$  oocytes (median: 9 oocytes; range: 1–53 oocytes). The distribution of the numbers of recovered oocytes was significantly different between the antagonist and long protocols ( $P = .0008$ ) (Fig. 1). In antagonist-stimulated cycles ( $n = 491$ ), most aspirations retrieved 6 to 10 (34%) or 1 to 5 (31%) oocytes. Following the long protocol, more aspirations resulted in  $\geq 16$  oocytes compared with antagonist protocol cycles (26% vs 13%, respectively).

Of the 654 cycles, oocytes were used for IVF in 23 patients; 10 who received the antagonist protocol and 13 who received the long protocol. Oocytes were used for ICSI in 631 patients; 454 who received the antagonist protocol and 177 who received the long protocol. Of all retrieved oocytes, 18 resulted in no embryo, 51 resulted in embryos that were not used for fresh embryo transfer, and 585 embryos were transferred on days 2 to 5 following fertilization. Clinical pregnancy was diagnosed in 232 (39.7%) transfer cycles, with 145 (24.8%) cycles resulting in 169 live babies: 122 singletons, 22 sets of twins, and 1 set of triplets. Two pregnancies with male fetuses ended in intrauterine fetal death. Among the 169 live babies, 50.9% were male, and this rate

**Table 1**

**Maternal characteristics.**

	Antagonist number	%	Long number	%	Total	
Age						% of 760
<30	186	34	93	43	279	37
30–34	156	29	77	35	233	31
35–39	159	29	45	21	204	27
40+	42	8	0	0	44	6
Missing	543	100	217	99	760	100
BMI						% of 56
Underweight	7	1	0	0.00	7	1
Normal	97	18	54	25	151	020
Overweight	196	36	79	37	275	036
Obese	240	44	83	38	323	43
Missing	540	100	216	100	756	100
Diagnosis	5		1			
$\geq 2$	89	16	58	27	147	19
Unexplained	116	21	34	16	150	20
Recurrent pregnancy loss (RPL)	1	0.00	1	0.00	2	0.00
Ovulation defect	77	14	19	9	96	13
PCOS	99	18	59	27	158	21
Tubal	54	10	14	6	68	9
Endocrine	6	1	24	11	30	4
Male factor	244	45	112	52	356	47
	597	110	263	121	860	

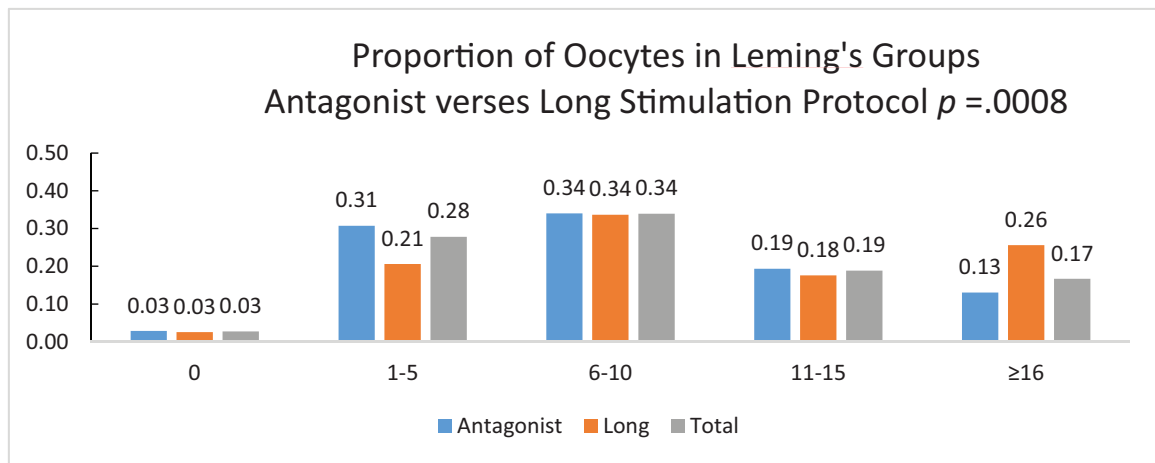


Figure 1. Distribution of the numbers of oocytes in the antagonist versus long protocol stimulation groups.

did not differ when comparing the antagonist (52.3%) with the long (48.3%) protocol groups ( $p=.740$ ) (Fig. 2). Only 8 completed pregnancies were the result of IVF; 2 of 8 (25%) in patients receiving the antagonist protocol and 6 of 11 (54.5%) in patients receiving the long protocol. The 137 completed pregnancies following ICSI included 91 pregnancies after 407 antagonist protocol transfers (22.4%) and 46 pregnancies following 159 long protocol transfers (28.9%;  $P=.125$ ). We summarized results from the ICSI group because 94.3% of our pregnancies resulted from ICSI. Data closely resembled that from the combined fertilization methods; that is, more babies from antagonist protocol-ICSI procedures were male ( $n=56$ ; 52.3%),

and more babies from long protocol-ICSI procedures were female ( $n=29$ ; 53.7%); however, the sex difference between these 2 groups was not statistically significant ( $P=.578$ ) (Fig. 3).

Other subgroup comparisons based on maternal age, number of embryos transferred, and day of transfer included widely variable numbers within groups, and differences between groups were also neither clinically nor statistically significant. For example, 532 cycles involved day 2 to 3 transfers, which resulted in 74 male babies (50.1%), while day 4 to 5 transfers were performed in only 52 cycles, with 23 resulting babies, of whom 12 were male (52.2%;  $P=.920$ ) (Fig. 4). The proportions of male babies by transfer day were also similar between protocols ( $P=.823$ ).

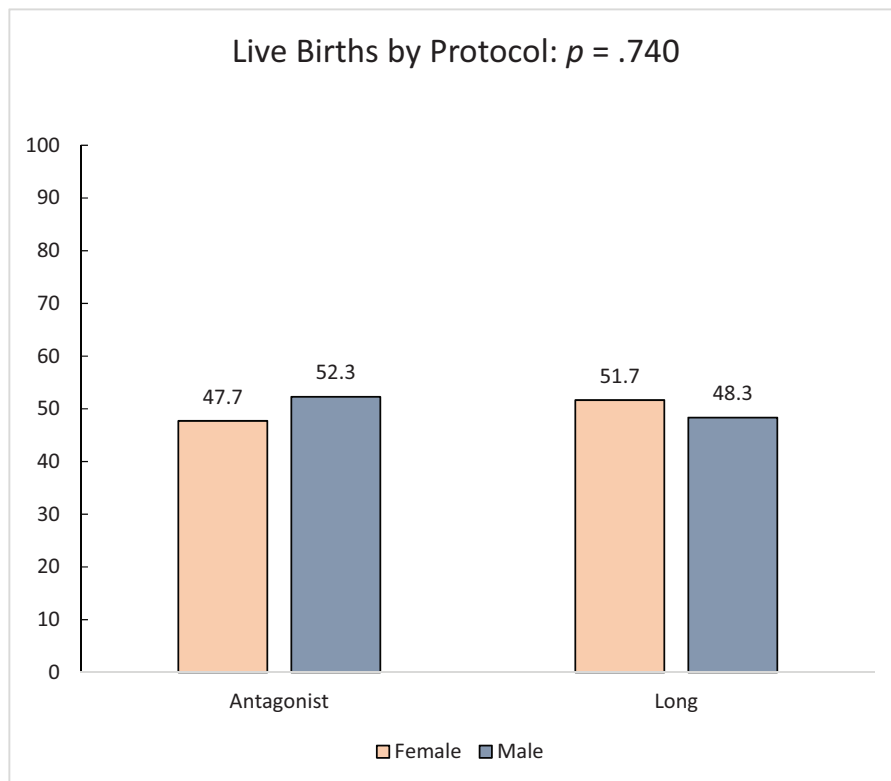
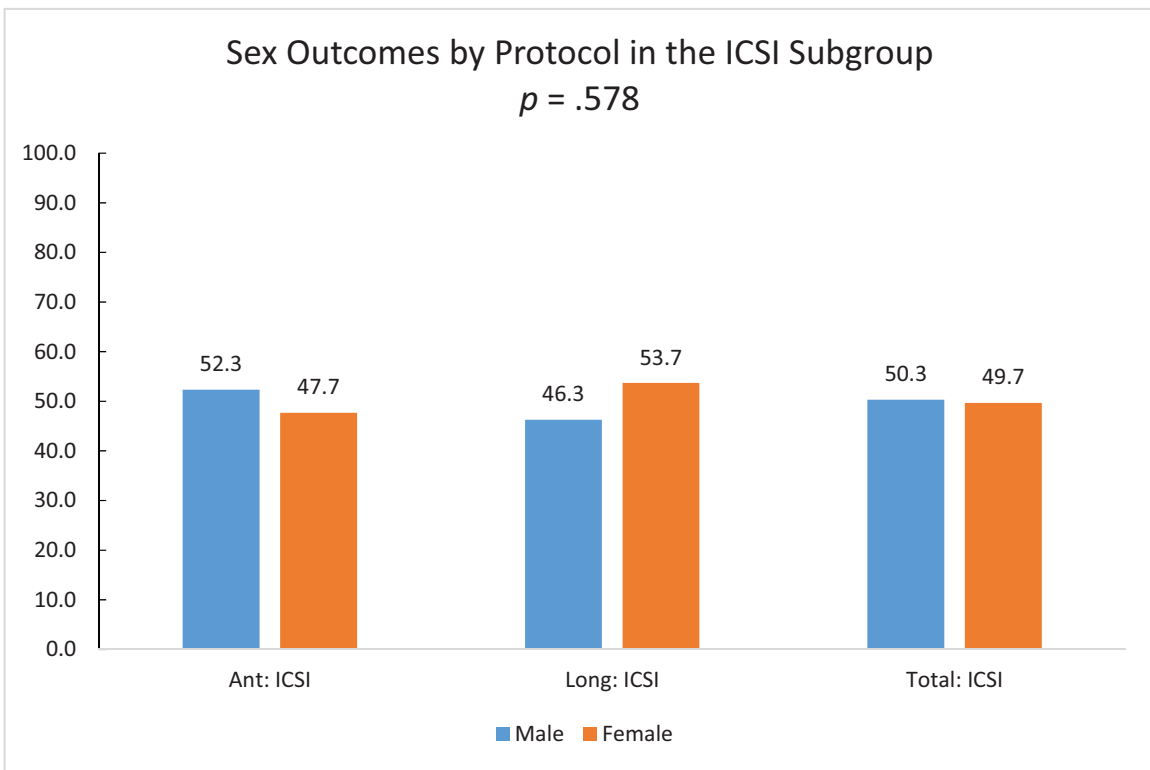


Figure 2. Live birth comparison following antagonist versus long-stimulation protocols.

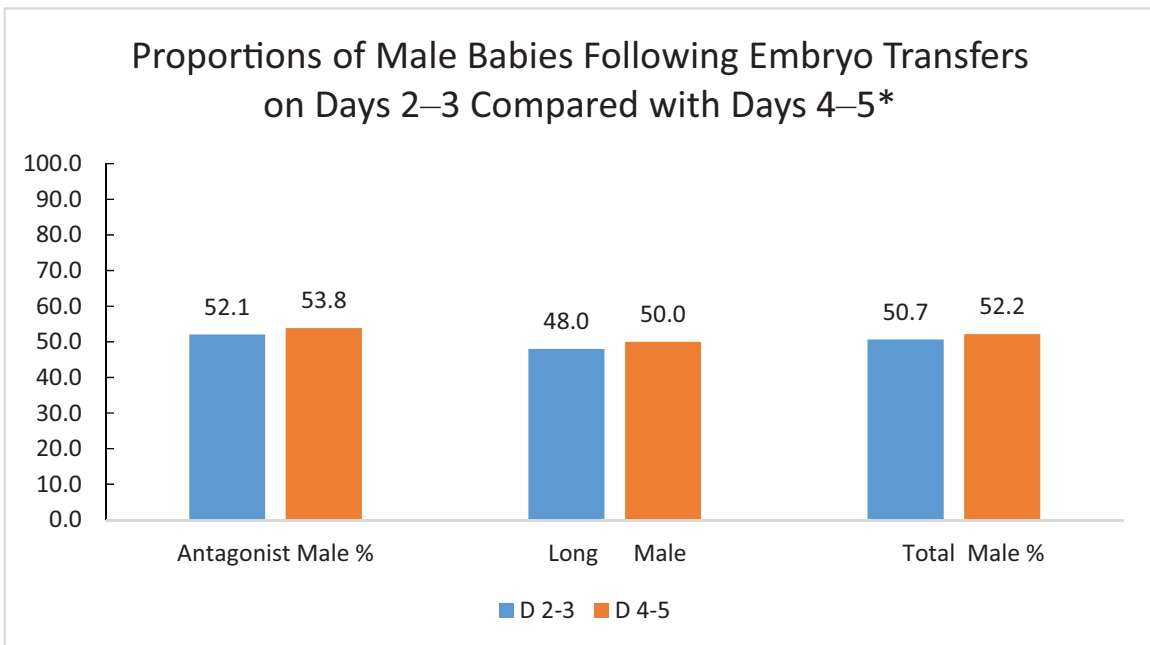


**Figure 3.** Sex outcomes by protocol in the ICSI subgroup. Ant=antagonist stimulation protocol, ICSI=intracytoplasmic sperm injection, Long=long stimulation protocol.

**4. Discussion**

A possible effect of ART procedures on offspring sex has been of interest since ART was introduced, clinically.<sup>[3]</sup> However, assessing a potential influence of ART on SSR is complicated by variability in previous observational studies, and among

investigated variables, minimal data are available for a possible effect of stimulation protocols on sex outcomes. A retrospective study of 7410 babies resulting from ART procedures between 2001 and 2015 in China included a sex comparison of 4332 babies resulting from long protocols with 2146 babies resulting



**Figure 4.** Proportions of male babies following embryo transfers on days 2 to 3 compared with days 4 to 5. \* =no significant, D=day.

from “other” protocols; male babies constituted 53.3% and 51.4% of each group, respectively ( $P=.146$ ). Similarly, we also found no apparent impact on the proportion of male babies between antagonist and long protocols in our, smaller, study.

The day of embryo transfer has received considerable attention regarding offspring sex. One report added their nonsignificant outcome from 205 deliveries to outcomes from 6 other reports comprising 86 to 365 babies from 2 clinics representing 1 to 2 years of experience.<sup>[4]</sup> The pooled results indicated significantly increased numbers of male babies after blastocyst transfer compared with cleavage-stage embryo transfer (57.3% vs 51.2%, respectively;  $P=.001$ ). A subsequent meta-analysis including 4 of these studies and a later report of 3 years’ experience at a single center comprising 1284 babies also concluded that significantly more male babies resulted from blastocyst transfer (odds ratio: 1.29; 95% confidence interval: 1.10–1.51).<sup>[5,6]</sup> Outcomes continue to be studied in single centers reporting hundreds of births, with data now also available from thousands of births recorded in ART registries. Of these reports, 3 single-center studies reported nonsignificant differences in male offspring proportions between blastocyst and cleavage-stage transfers.<sup>[7,8]</sup> However, 1 study reported a greater proportion of male babies following the transfer of cleavage-stage embryos.<sup>[7]</sup> Although both protocols in our study resulted in more male babies when transfers were performed after day 4 or 5 compared with day 3 or earlier, our numbers were too small to have statistical significance.

Certain previous studies with up to 15-year data from several thousand procedures report increased male babies following blastocyst transfer compared with cleavage-stage embryo transfer. If this skewed outcome bias for male babies has been stable despite refinements and changes in protocols and technology, a dominant and resilient embryonic or maternal process may be preferentially facilitating blastocyst development by male embryos or survival in utero of male blastocysts. Several mechanisms have been proposed to explain this phenomenon,<sup>[9]</sup> however, studies have rejected theories that male embryos cleave faster and have better morphology, thereby increasing their likelihood of selection for transfer. In fact, a study comparing live births following a single poor-quality embryo transfer ( $n=54$ ) compared with a single good-quality embryo transfer ( $n=386$ ) reported more male babies in women receiving a poor-quality embryo; however, the difference was not statistically significantly different (53.7% vs 44.8%, respectively;  $P=.35$ ).<sup>[10]</sup> One theory suggests that precocious X-chromosome inactivation in vitro, combined with ICSI-induced reduced trophectoderm cells in female blastocysts, may result in selective female mortality early postimplantation, contributing to the sex ratio variations seen in ART cycles.<sup>[9]</sup>

A large Australian study reported more male babies following blastocyst transfer compared with cleavage-stage embryo transfer following both IVF (56.1% vs 51.5%, respectively) and ICSI (52.5% vs 48.7%, respectively).<sup>[11]</sup> One Chinese study reported 52.3% of 79 606 babies born after IVF were male, compared with 49.7% of 31 276 babies born after ICSI ( $P<.001$ ).<sup>[12]</sup> Another Chinese study reported more male babies after IVF ( $n=5381$ ) compared with ICSI ( $n=1111$ ), but the difference was not statistically significant (53.2% vs 50.4%, respectively;  $P=.10$ ).<sup>[2]</sup> Data from a UK national registry of over 100,000 babies also noted significantly more male babies after IVF compared with ICSI (52.1% vs 49.3%, respectively;  $P<.0001$ ).<sup>[3]</sup> A small study of 38 monozygotic twins reported a 2.2 male:female sex ratio following IVF compared with a 0.58 ratio following ICSI (69.0% vs 36.8% male babies, respectively;  $P=.004$ ).<sup>[13]</sup> A Norwegian study of 420 babies reported 55%

males following day 5 transfer of IVF embryos, compared with 41% male babies following day 5 transfer of ICSI embryos.<sup>[14]</sup> Our ICSI subgroup, which comprised the majority of our completed pregnancies, had reverse sex prominence in completed pregnancies following the antagonist protocol compared with the long protocol. Although this difference was not statistically significant, our ICSI antagonist outcome of 52.3% male babies is consistent with that reported for blastocyst-stage ICSI embryos in the study by Dean et al<sup>[11]</sup> of 52.5% male babies, while our finding was 46.3% male babies in the ICSI long-protocol group.

Factors affecting sex other than the fertilization method and transfer day, including semen, sperm, and embryo quality characteristics; transfer of fresh versus frozen embryos; number of embryos transferred; body mass index; and various combinations and subgroups prepared from these and other variables have been described. Other studies investigated the causes of sex ratio differences in the general population, including stress, pollution, and time required to achieve pregnancy, any of which may also apply to women undergoing ART.<sup>[15–19]</sup> As the number of contributing variables expands, it becomes increasingly difficult to envision a regression equation that could be used to predict or increase the chance of having a chosen offspring sex. Regardless, possible public health concerns could result if ART does have an effect on sex outcomes.<sup>[3]</sup> The authors of the Australian study reporting proportions of male offspring ranging from 56.1% following IVF blastocyst transfer to 48.7% following ICSI cleavage-stage embryo transfer recommended that fertility clinics and patients become aware of the potential increase in IVF blastocyst transfer, and a potential decrease following ICSI cleavage-stage embryo transfer.<sup>[11]</sup> This concern was assessed using a model of constrained sex allocation, which predicted that to have a detectable effect on SSR in society, over 20% of couples would have to use ART.<sup>[20]</sup> This proportion is greater than the estimated prevalence of human fertility issues. However, reproduction continues to be a popular topic in the media, and the study by Dean et al<sup>[11]</sup> resulted in a British Broadcasting Corporation online news article titled, “Women using IVF to get pregnant should be aware that they will be more likely to have a boy than a girl, say experts.”<sup>[21]</sup> These statements need to be interpreted carefully and we need to be prepared to discuss these media publications with our patients, and to provide the appropriate information.

Our ART patients wish to have a successful pregnancy, and they will choose an identified procedure that increases that success. Accordingly, it is important to continue researching factors affecting embryo development for both sexes from oocyte aspiration through delivery, and implement new improvements in ART technology.

As a limitation of our study, our small sample size did not provide sufficient power to detect differences between groups for sex outcomes for the factors that we analyzed, including our comparison between antagonist and long protocols. We realize the importance of having a robust database that can be easily summarized to monitor trends, and continued periodic outcome assessment is planned. As we review data from other countries, we are aware that Saudi Arabia needs an ART registry, from which individual Saudi Arabian centers can compare their data within the country and with other countries’ registries.

## Acknowledgments

The authors thank Miss Valerie Zimmerman for proofreading our manuscript and Miss Ouhood Kaddour for her initial input on the study.

## Author contributions

**Conceptualization:** Dania Al-Jaroudi.

**Data curation:** Dania Al-Jaroudi, Gamar Salim, Saeed Baradwan.

**Formal analysis:** Dania Al-Jaroudi, Saeed Baradwan.

**Investigation:** Dania Al-Jaroudi.

**Methodology:** Dania Al-Jaroudi, Gamar Salim.

**Project administration:** Dania Al-Jaroudi.

**Software:** Dania Al-Jaroudi, Gamar Salim.

**Supervision:** Dania Al-Jaroudi.

**Validation:** Dania Al-Jaroudi, Saeed Baradwan.

**Visualization:** Dania Al-Jaroudi, Saeed Baradwan.

**Writing – original draft:** Dania Al-Jaroudi, Gamar Salim, Saeed Baradwan.

**Writing – review & editing:** Dania Al-Jaroudi, Saeed Baradwan.  
Dania Al-Jaroudi orcid: 0000-0003-3612-8199

## References

- [1] Ethics Committee of the American Society for Reproductive Medicine. Preconception gender selection for nonmedical reasons. *Fertil Steril* 2015;103:1418–22.
- [2] Chen M, Du J, Zhao J, et al. The sex ratio of singleton and twin delivery offspring in assisted reproductive technology in China. *Sci Rep* 2017; 7:7754.
- [3] Barsky M, St Marie P, Rahil T, et al. Are perinatal outcomes affected by blastocyst vitrification and warming? *Am J Obstet Gynecol* 2016;215: 603.e1-603.e5.
- [4] Milki AA, Jun SH, Hinckley MD, et al. Comparison of the sex ratio with blastocyst transfer and cleavage stage transfer. *J Assist Reprod Genet* 2003;20:323–6.
- [5] Chang HJ, Lee JR, Jee BC, et al. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. *Fertil Steril* 2009;91:2381–90.
- [6] Luna M, Duke M, Copperman A, et al. Blastocyst embryo transfer is associated with a sex-ratio imbalance in favor of male offspring. *Fertil Steril* 2007;87:519–23.
- [7] Lin TK, Su JT, Lee FK, et al. Cryotop vitrification as compared to conventional slow freezing for human embryos at the cleavage stage: survival and outcomes. *Taiwan J Obstet Gynecol* 2010;49:272–8.
- [8] Zhu J, Zhuang X, Chen L, et al. Effect of embryo culture media on percentage of males at birth. *Hum Reprod* 2015;30:1039–45.
- [9] Tarín JJ, García-Pérez MA, Hermenegildo C, et al. Changes in sex ratio from fertilization to birth in assisted-reproductive-treatment cycles. *Reprod Biol Endocrinol* 2014;12:56.
- [10] Oron G, Sokal-Arnon T, Son WY, et al. Extended embryo culture is not associated with increased adverse obstetric or perinatal outcome. *Am J Obstet Gynecol* 2014;211: 165.e1–165.e7.
- [11] Dean JH, Chapman MG, Sullivan EA. The effect on human sex ratio at birth by assisted reproductive technology (ART) procedures—an assessment of babies born following single embryo transfers, Australia and New Zealand, 2002–2006. *BJOG* 2010;117: 1628–34.
- [12] Bu Z, Chen ZJ, Huang G, et al. Live birth sex ratio after in vitro fertilization and embryo transfer in China—an analysis of 121,247 babies from 18 centers. *PLoS One* 2014;9:e113522.
- [13] Al Dibouni Z, Shah T, Wheat S, et al. Incidence, sex ratio and perinatal outcomes of IVF and ICSI monozygotic twin pregnancies following either cleavage or blastocyst stage embryo transfer. *Hum Genet Embryol* 2016;6:132.
- [14] Hentemann MA, Briskemyr S, Bertheussen K. Blastocyst transfer and gender: IVF versus ICSI. *J Assist Reprod Genet* 2009; 26:433–6.
- [15] James WH, Grech V. Homosexual sons and the fraternal birth order effect: the role of elevated maternal intrauterine testosterone. *Arch Sex Behav* 2017;47:33–6.
- [16] Bonde JP, Wilcox A. Ratio of boys to girls at birth. *BMJ* 2007;334: 486–7.
- [17] Kaitz M, Rokem AM, Mankuta D, et al. Exposure to childhood traumas ups the odds of giving birth to daughters. *Arch Womens Ment Health* 2014;17:159–66.
- [18] McDonald SA, Qendri V, Berkhof J, et al. Disease burden of human papillomavirus infection in the Netherlands, 1989–2014: the gap between females and males is diminishing. *Cancer Causes Control* 2017;28:203–14.
- [19] Smits LJ, de Bie RA, Essed GG, et al. Time to pregnancy and sex of offspring: cohort study. *BMJ* 2005;331:1437–8.
- [20] Hardy IC, Maalouf WE. Partially constrained sex allocation and the indirect effects of assisted reproductive technologies on the human sex ratio. *J Biosoc Sci* 2017;49:281–91.
- [21] Roberts M, 2010. *IVF 'increases the chance of having a baby boy'* [online]. British Broadcasting Corporation. Available at: <http://www.bbc.com/news/health-11419466>. [Accessed November 7, 2017].