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Research Article

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Does Assisted Reproductive Technology have an Effect on Human Secondary Sex Ratio?

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Abstract

Assisted reproduction technology (ART) is up-trending. Therefore, the number of babies born by ART is increasing. ART may influence the secondary sex ratio (SSR) compared to natural conception. to evaluate the effect of ART on SSR. a pilot retrospective medical records review study was conducted on 201 live-birth babies born to couples who attended Albayda Fertility Centre. 97 babies were born by ART procedures [intrauterine insemination (IUI) and intracytoplasmic sperm injection (ICSI)], and the remaining were born by non-ART (medical treatment or post-hysteroscopy). The overall SSR was 46%, and the SSR for non-ART babies was (54.8%) higher than that for the ART offspring (47.4%). However, the difference was non-significant. More girls were born as a result of ICSI (SSR = 43.7%). In contrast, more males were born following IUI (SSR = 47.7%). But the difference was insignificant. Regarding the stage of embryo transfer (ET) in babies born by ICSI, a higher, but non-significant difference (Fisher's Exact test = 0.9) was found in the blastocyst stage than in the cleavage stage. ART might cause a bias in sex ratio at birth, and this change in SSR was found to be affected by the ART procedure applied. The mechanism of these effects is still controversial, and larger multi-centric studies are still warranted.

Keywords: ART, Natural Conception, SSR, IUI, ICSI, Cleavage Stage Embryo Transfer, Blastocyst Embryo Transfer.

INTRODUCTION

Assisted reproductive technology (ART) to treat infertility has developed rapidly since the first in-vitro-fertilization (IVF) baby was born in 1978 (Dean et al., 2010). The number of babies born from ART is increasing rapidly. Worldwide, approximately five million babies have been born as a result of ART (Sandin et al., 2013).

Primary sex ratio (PSR) is the ratio after conception, defined according to the number of ova fertilized by X or Y-bearing spermatozoa (Wang et al., 2010). The ratio at birth is known as the secondary sex ratio (SSR); it is the ratio of male births to total births (Chen et al., 2017; Dean et al., 2010). Others defined SRR as the ratio of male live births to 100 female live births (Bu et al., 2014). The primary sex ratio was found to range between 107 and 170 males / 100 female live births (Bonduelle et al., 2002), whereas the crude secondary sex ratio was estimated as 51.3% (Dean et al., 2010; Wang et al., 2010). This drop in the natural conception SSR is at-

tributed to several environmental and biological factors, such as maternal age, metabolic diseases in women, stressors (war, earthquake), toxins (smoking, pollutants, and pesticides), and race (Rueness et al., 2012). There are three assisted reproductive techniques (ART): intrauterine insemination (IUI), intracytoplasmic sperm injection (ICSI), and in vitro fertilization (IVF). These ART methods not only differ technically but also may have different effects on SSR (Dean et al., 2010).

These ART methods may affect SSR compared to natural conception as the gametes were exposed to external influences during ART, which might vary from the effect of internal factors during natural pregnancy (Chen et al., 2017). Whether the difference in SSR among ART babies results from natural causes (biological or environmental reasons) or as a result of ART per se is still controversial (Dean et al., 2010).

Thatcher et al. found that the SSR of live-born offspring following in-vitro fertilization/embryo transfer (IVF /ET) was significantly higher than normal (64.1%) (Thatcher et al., 1989), whereas in 2000 Ghazzawi and his colleagues reported that a higher percentage of female babies (61.7%) were born following the transfer of embryos fertilized by intracytoplasmic sperm injection (ICSI) (Ghazzawi et al., 2000). A higher SSR in babies born after IUI and a lower SSR after ICSI was reported. This reduction in SSR following ICSI could be explained by the use of ICSI, which is dominantly indicated in the management of male factor infertility (Luke et al., 2009). IUI is the first line of infertility treatment before proceeding to more invasive and expensive procedures such as IVF and ICSI (Dean et al., 2010). Different trials have been undertaken for separating Y- and X-bearing spermatozoa based on the motility of the sperms, but controversial results have been reported (Supramaniam et al., 2019). No direct evidence was found that the sperm preparation procedures for separating motile spermatozoa to be used for either IUI or IVF would lead to an imbalance of male and female babies from the norm (Javed et al., 2019; Yan et al., 2006).

(Fernando et al., 2012) reported that SSR in assisted reproduction technology (ART) babies born from ICSI procedures is lower than those conceived with IVF (Fernando et al., 2012). Dean et al. (2010) demonstrated that the day of embryo transfer (the stage of embryonic development during an ART cycle) also had an effect on the SSR, with a higher proportion of male births reported following a blastocyst stage transfer when compared with dividing stage embryo transfer, and this was found to be independent of the fertilization method (IVF or ICSI) (Dean et al., 2010).

The stability of the secondary sex ratio is of great importance in preserving social stability and improving economic development as happening worldwide. In Libya, more babies were born as a result of ART. To our knowledge, no research has assessed SSR in Libya, and therefore, we sought to evaluate the influence of assisted reproductive techniques on SSR to babies delivered within Albayda Fertility Center to confirm or rule out the effect of assisted reproductive techniques on the secondary sex ratio.

MATERIALS AND METHODS

A pilot retrospective medical records review study was conducted on 201 live-birth offspring born after different modalities of treatment to infertile couples who attended Albayda Fertility Centre/Libya from 2020 to 2022. The subjects included singletons and twins (none was monozygotic), and only one mother gave birth to triplets.

This study was conducted to evaluate the potential influences of ART on the secondary sex ratio (live-birth male to total live-birth). The data variables included in the study were: the mother's and father's age at conception, cause of infertility, type and duration of infertility, how the mother got pregnant, and the baby's gender at birth. The gender of the included babies in the current study was not subject to the sex selection process. The semen samples used in IUI were fresh, and the samples were prepared by swim-up technology for both IUI and ICSI. The embryos transferred in ICSI either in cleavage or blastocyst stage were fresh (no frozen embryos).

The included live-birth babies were divided into two groups: the ART group (babies resulting from IUI and ICSI) and the non-ART group (babies of women who got pregnant by lifestyle management, medical treatment, or pregnancy after hysteroscopy). ART group was divided into IUI and ICSI subgroups, and the ICSI subgroup was further divided according to the embryonic stage at embryo transfer (cleavage stage and blastocyst stage).

Statistical analyses

Statistical analyses were performed using SPSS-26. The continuous data were presented as mean and standard deviation, and Student t-test was used to compare the data. Categorical data were represented as numbers and proportions, and the Chi-square statistic test was used to compare the categorical data. The level of significance was considered at a P value < 0.05.

RESULTS

Two hundred and one live-birth babies delivered to Libyan infertile couples were included in the study. 55.8% of the included couples were presented with primary subfertility and 44.3% presented with secondary subfertility (Figure 1). The duration of infertility ranged between one and fourteen years. The mean age of the wives and husbands was 30.4 (5.2) and 37 (6.3) years respectively. Most of the women (98%) had no antenatal or postnatal complications, and only three mothers had their pregnancy complicated with pre-eclampsia.

Most of the newborns (91.5%) were term, and only 17 (8.5%) were preterm (less than completed 37 gestational weeks) The birth weight ranged between one and four kilograms, and the mean birth weight was 2.8 (0.4) Kg. Neonatal complications occur only in 16 cases (7.9 %), neonatal death in twelve newborns, and most of them are preterm, and four have congenital anomalies.

Table: (1). Demographic data of ART& non-ART group

Variable		ART	Non-ART	Test of significance	*P*
Maternal age		29.8 (4.5)	31 (5.7)	*t*= 1.6	0.09
Paternal age		36.4 (5.9)	37.8 (6.6)	*t*= 1.5	0.1
Infertility type	Primary	63	49	X^2 = 6.4	^{FE}0.01*
	Secondary	34	55		
Infertility duration		4 (2.4)	3.7 (2.5)	*t*= 1.1	0.3
Infertilit aetiology	Female	68	68	X^2 = 4.5	^{FE}0.2
	Male	9	10		
	Combined	3	11		
	Unexplained	17	15		
Gestational age	Term	87	97	X^2 = 0.8	^{FE}0.45
	Preterm	10	7		
Birth weight		2.8 (0.5)	2.9 (0.4)	*t*= 1.7	0.2
Neonatal complications		12	4	X^2 = 4.9	^{FE}0.03*
Maternal complications		2	1	X^2 = 1.3	^{FE}0.4

X2: Chi square test FE: Fisher's exact test

*: Statistically significant at p≤ 0.05

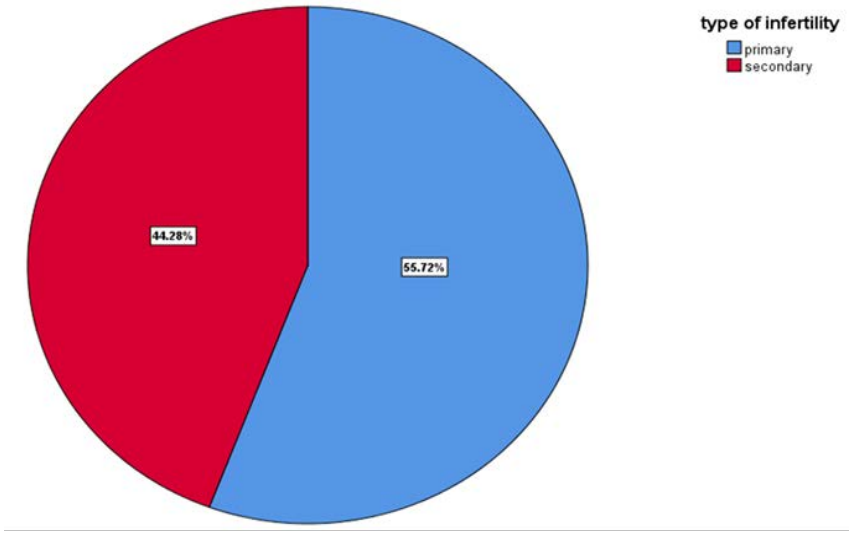

Figure: (1). type of infertility of the included couples

Out of two hundred and one live births during the period of study, 97 newborns were delivered as a result of IUI and ICSI (ART group), and 104 newborns as a result of medication or following hysteroscopy (non-ART group).

Comparing the ART with the non-ART group:

Regarding maternal and paternal age, infertility duration, gestational age, and birth weight, the difference did not reach the significant level between the two studied groups. Primary infertility was significantly more frequent in the ART group than in the non-ART group (p=0.01). Neonatal complications were found to be more significant in the ART newborns (p=0.03) (Table 1). The causes of infertility were due to female problems in most of the cases, followed by unexplained infertility, then male-related causes, and the least was due to combined male and female causes. However, the difference was non-significant between the ART and non-ART groups regarding the causes of infertility (Figure 2).

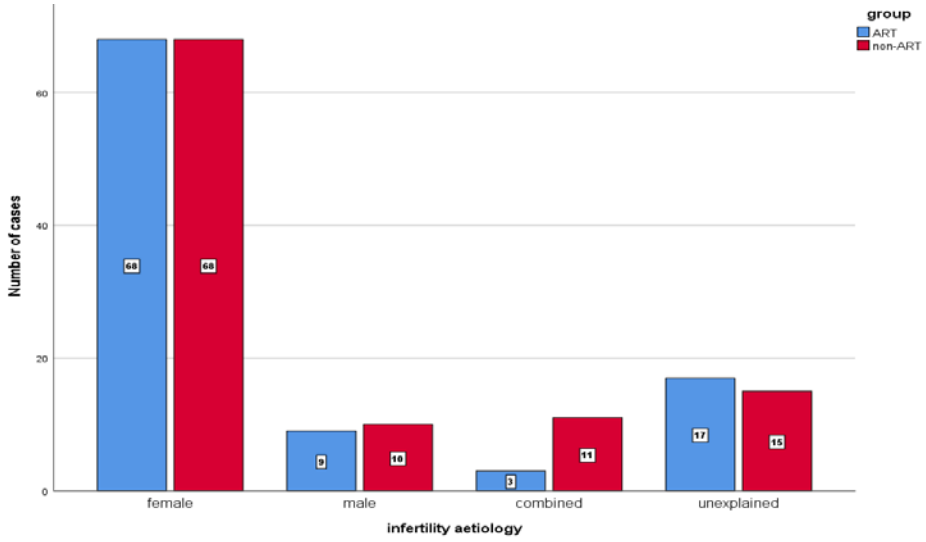

Figure: (2). causes of infertility in ART and non-ART group

The secondary sex ratio (SSR) for all the included newborns was (93/201= 46%). The SSR for ART newborns (46/97=47.4%) was lower than that for the non-ART newborns (57/104=

54.8%), but the difference was not significant (p = 0.325) (Figure 3& Table 2).

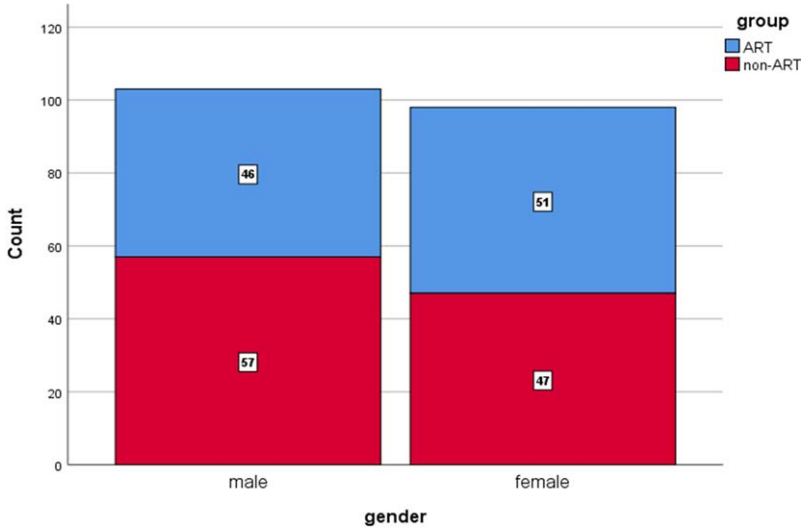

Figure: (3). SSR in the Art and non-ART group

Table: (2). SSR for ART and non-ART newborns

	Male	Female	SSR	Test of significance	*P*
ART	46	51	47.4%	X^2= 1.1	^{FE}0.3
Non-ART	57	47	54.8%		

In the ART group, SSR for IUI 47.7% was higher than SSR for ICSI (43.7%), but the difference was not significant P = 0.83 (Table 3). With regard to the embryonic stage of development of the embryo within the ICSI sub-group, SSR for the blastocyst stage (54%) was higher than that for the cleavage stage of the embryo transfer (45%). However, the difference was non-significant (Fisher's Exact test = 0.9) (Figure 4).

Table: (3). Comparison of SSR between IUI and ICSI

	Male	Female	SSR	Test of significance	*P*
IUI	31	34	47.7%	X^2= 1.3	^{FE}0.83
ICSI	14	18	43.7 %		

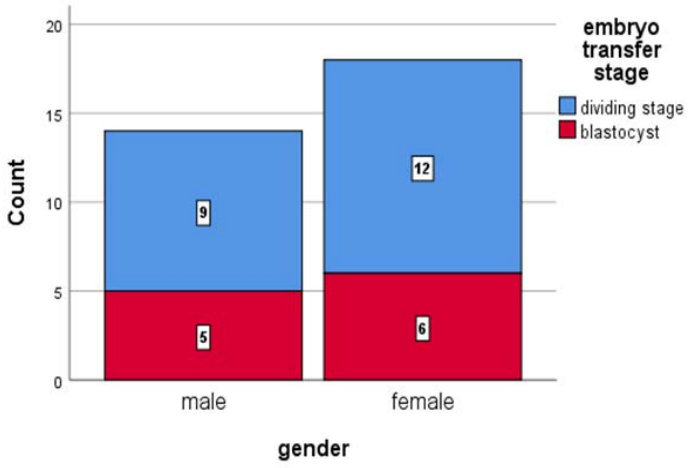

Figure: (4). SSR within ICSI subgroup according to the stage of embryo transfer

DISCUSSION

A pilot retrospective medical records review study was conducted during the period from January 2020 to December 2022 at Albayda Fertility Center on 201 offspring delivered after different modalities of treatment of infertile couples and analyzed the effect of assisted reproductive technique (ART) on the gender of newborns (SSR).

In the current study, SSR for the whole study population (ART and non-ART babies) was 46%, and this result was lower than what was reported (51.22%) in a previous study (James, 2012). This could be explained by the small sample size, geographical differences, or demographic structure of the participants. In this study, there was no conventional IVF, and it was reported that the SSR after standard IVF was actually higher than that for the population sex ratio, and also following ICSI (Bonduelle et al., 2002; Källén et al., 2005; Wang et al., 2020).

A study from the United Kingdom reported the SSR of babies born through ART to be 50.98% (Supramaniam et al., 2019). Chinese multi-centric research from 18 reproductive centers found that the SSR was 51.8% and concluded that ART may affect the SSR (Bu et al., 2014). In the present study, the SSR for the ART group (IUI & ICSI) was 47.4% lower than that for the non-ART group (SSR= 54.8%). In contrast, a previous study (Hann et al., 2018) compared the ART offspring with the babies born after natural pregnancy. They reported a similar SSR between the two sets of babies, suggesting that ART did not affect SSR.

In this study, SSR is higher in pregnancies achieved by IUI (47.4%) compared to that by ICSI (43.7%), but the difference was not significant. This result was in accordance with a previous study (Maalouf et al., 2014).

Increased SSR in IUI and IVF (closer to natural conception) more than that for ICSI, as in these methods (IUI and IVF), the sperms compete for fertilization, while in ICSI, the fertilizing sperms selected by embryologists (Maalouf et al., 2014) and so the natural selection of sperm is bypassed. Also, the gametes in ICSI are exposed to more manipulation than in IUI and IVF (Chen et al., 2017). The reduced SSR after ICSI compared to IUI and natural pregnancy could be explained by the reduced number of Y-bearing spermatozoa in a male partner, as ICSI is greatly indicated in the treatment of male factor infertility that is frequently associated with poor spermatogenic function (Ménézo, 2006; Tarín et al., 2014). All these could explain the higher SSR in IUI than in ICSI group in the current study.

Regarding the stage of embryo transfer; this study revealed that embryo transfer at a blastocyst stage results in SSR (54%) higher than after early stage (dividing) embryo transfer. A similar study (Dean et al., 2010; Majeed et al., 2019; Supramaniam et al., 2019) revealed that a predominance male gender is seen with blastocyst stage embryo transfers with IVF or ICSI (Majeed et al., 2019). In contrast, other studies concluded that blastocyst transfer was not associated with an increased SSR (Al Dibouni et al., 2016; Milki et al., 2003; Żądzińska et al., 2011). Blastocyst stage embryo transfers were thought to lead to a higher proportion of males, secondary to the quick growth potential of male embryos due to their higher ability of pyruvate and glucose uptake compared to female counterparts (Bonduelle et al., 2002; Ray et al., 1995).

ART has been a newly employed technology in Libya in recent years, and therefore, the sample size was relatively small. A larger multi-centric study will be more informative.

CONCLUSION

ART might alter the secondary sex ratio. This change in the sex ratio at birth was found to be affected by the ART procedure used. The mechanism of these effects is still controversial, so larger and multi-centric studies are still warranted.

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ETHICS

There are no ethical issues regarding the current research.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

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