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Original article

An ICSI rate of 90% minimizes complete failed fertilization and provides satisfactory implantation rates without elevating fetal abnormalities

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ABSTRACT

IVF cycles utilizing the ICSI technique for fertilization have been rising over the 25 years since its introduction, with indications now extending beyond male factor infertility. We have performed ICSI for 87% of cases compared with the ANZARD average of 67%. This retrospective study reports on the outcomes of 1547 autologous ART treatments undertaken over a recent 3-year period. Based on various indications, cases were managed within 3 groupings - IVF Only, ICSI Only or IVF-ICSI Split insemination where oocytes were randomly allocated. Overall 567 pregnancies arose from mostly single embryo transfer procedures up to December 2016, with 402 live births, comprising 415 infants and a low fetal abnormality rate (1.9%) was recorded. When the data was adjusted for confounders such as maternal age, measures of ovarian reserve and sperm quality, it appeared that IVF-generated and ICSI-generated embryos had a similar chance of both pregnancy and live birth. In the IVF-ICSI Split model, significantly more ICSI-generated embryos were utilised (2.5 vs 1.8; $p < 0.003$) with productivity rates of 67.8% for pregnancy and 43.4% for livebirths per OPU for this group. We conclude that ART clinics should apply the insemination method which will maximize embryo numbers and the first treatment for unexplained infertility should be undertaken within the IVF-ICSI Split model. Whilst ICSI-generated pregnancies are reported to have a higher rate of fetal abnormalities, our data is consistent with the view that the finding is not due to the ICSI technique per se.

1. Introduction

The intra-cytoplasmic sperm injection (ICSI) technique was heralded as a distinct advance in the field of assisted reproductive technology (ART) as it enabled improved outcomes over the conventional in-vitro fertilization (IVF) procedure for those cases categorized as male-factor infertility [1]. However, concern was expressed as the selection technique for the individual spermatozoon to be injected, bypasses many natural biological processes that are thought to minimise the rate of embryonic and subsequent fetal anomalies. Such sperm-related natural conception processes include capacitation, hyper-activated motility, the acrosome reaction, cumulus dispersal, oocyte-induced sperm activation, zona-binding and sperm-egg membrane interaction causing cortical granule release [2]. However, the injection of a single spermatozoon immobilized by mechanical fracture of its tail, by-passes all of the "molecular passport" hurdles [3]. Hence the rational consideration prevailing to recent times was to avoid ICSI whenever it was deemed unnecessary. The pre-ICSI era data for fetal abnormalities showed higher rates for IVF infants, than those naturally conceived [4,5]. However, current data has seen this difference disappear, to be replaced by the ICSI-generated infants showing abnormality rates of 7.1% compared with 4.0% in the general population as well as for IVF-generated children [6]. Conversely, data from South Australia showed elevations for both IVF and ICSI against natural conceptions, but intriguingly this was only for younger women (< 30 years; OR 1.42); the difference disappearing for older women (35–39 years; OR 1.01) and actually reversing for women ≥40 years conceiving (most cases utilising ICSI; with OR 0.45; 95% CI 0.22–0.92) [7].

Despite the theoretical concerns, over the 25 years since its application, "current evidence suggests no difference in perinatal outcomes or congenital malformation risks in ICSI children when compared to naturally conceived children" [8]. Where elevated rates have been reported, such studies appear to have been affected by various confounders (including patient factors, ART confounders and study biases).

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Being of the view that ICSI is a safe procedure, our group has sought to extend its application outside male-factor and into the generally accepted non-male factor situations. These include paternal infections including HIV (to minimise the risk of disease transmission), preimplantation genetic diagnosis or screening (to exclude DNA contamination), as well as in donor programmes utilizing frozen spermatozoa or oocytes (to maximize fertilization opportunity in precious circumstances) [9,10].

To this date, a high specificity diagnostic test to predict optimal fertilization by IVF does not exist. A normal semen analysis profile according to WHO standards [11] has universally accepted limitations, and the widely adopted DNA fragmentation testing techniques have also fallen short on the anticipated expectations, despite providing some improvement in directing cases towards ICSI insemination [12]. Other techniques such as the hamster oocyte penetration test as well as a test of sperm binding to donor oocytes have met with significant ethical challenges in many countries, including Australia.

Having identified that DNA fragmentation testing also has limitations in predicting the likelihood of reduced fertilization of oocytes, even complete fertilization failure, we introduced the idea that all new cases entering ART programmes should be offered an ICSI-IVF Split fertilization "test", in order to diagnostically determine the optimum fertilization mode and to ensure the chance of fertilization for at least some eggs. Where IVF embryos were generated, this offered the opportunity for selecting such for embryo transfer (ET) if deemed of suitable quality. This proposal followed from documented cases of unexpected complete fertilization failure in couples with normal semen parameters and thus caused us to expand our indications for ICSI accordingly [13]. Ultimately, this has led to our clinic undertaking ICSI in 87% of patients where the overall rate for Australia and New Zealand currently stands at 67% [14].

This study reports on the outcomes of adopting a policy of offering all new couples with unexplained or poorly explained infertility, an IVF-ICSI Split protocol as a diagnostic exercise, thereafter applying IVF Only if that was clearly demonstrated to be optimal. The ICSI Only protocol applied for established indications, mainly male factor infertility but also included a range of other female considerations such as advanced age and low ovarian reserve. The outcomes of interest were to optimize the number of usable embryos which can be derived from a single oocyte pickup procedure (OPU) and the subsequent pregnancy and live births arising from those embryos; both fresh and frozen.

2. Materials and methods

2.1. Indications for ICSI

2.1.1. Male infertility factors

Most cases deemed to have a severe male factor were allocated to ICSI, although on occasion some severe male factor couples requested IVF or IVF-ICSI Split to determine if any IVF embryos could be created. The main male infertility factors for ICSI allocation included semen abnormalities, high rates of DNA fragmentation, males of advanced age and patients requiring surgical retrieval of sperm (7.4% of ICSI cases) including Vasal flush, MESA, PESA, TESA or micro-TESE (microsurgical epididymal sperm aspiration, percutaneous sperm aspiration, testicular sperm aspiration or microsurgical testicular sperm extraction, respectively).

The two sperm evaluation methods applied included semen analysis according to WHO criteria [11] and the DNA fragmentation index according to the Halo Test [15] or sperm chromatin structure assay; SCSA [16,17]. The primary criterion for deeming "significant male factor" related to the finding of diminished normal sperm morphology < 4% or a DNA fragmentation index of 15% (reduced from 30% following a research study at PIVET [13]). The fertilization chances with ICSI are improved where DNA fragmentation is shown, but high levels of fragmentation can compromise even the ICSI rates [17].

Other male medical conditions such as obesity [18,19], varicocele [20] and those with male reproductive tract issues such as past testicular trauma, antisperm antibodies, maldescent of testes, history of torsion, orchidopexy or even the isolated finding of reduced volume testes [21] were considered for ICSI. In addition, males with chronic disease especially involving chemotherapy or radiotherapy, or those with infectious disease such as HIV, Hepatitis B and Hepatitis C were also advised to use ICSI insemination. Finally, those on drug therapy which may affect fertilizing capacity such as sulphasalazine, cimetidine, allopurinol amongst others [22], along with males that use recreational drugs or are exposed to chemicals and heavy metals in "risky" occupations such as welding, were also advised ICSI.

2.1.2. Female factor

Advanced female age as well as reduced ovarian reserve are interconnected to lower antral follicle counts (AFCs) and low serum anti-Mullerian hormone (AMH). These combinations are closely associated with poor IVF prognosis, which includes an array of deficiencies such as reduced fertilization of oocytes and low oocyte numbers on retrieval. ICSI can at least improve oocyte fertilization, although it may not have any major influence over embryo quality or implantation potential. It does however reduce the problem of polyspermic IVF fertilization seen more frequently in oocytes from women of advanced age or diminished ovarian reserve [23,24].

It was reported by Diedrich's group that where < 4 oocytes are recovered, ICSI guarantees a successful treatment outcome more often than IVF and encourages the idea of milder forms of stimulation [25]. Our clinic has adopted a milder stimulation policy in recent years such that many women will now generate < 5 oocytes and ICSI provides a greater chance of generating embryos for transfer, especially if few oocytes [26] or only a single oocyte is retrieved [27].

Finally, oocyte anomalies are also linked to advanced female age and poor prognosis cases. Zona thickening is associated with advanced maternal age and zona hardening is associated with cryopreservation, especially for immature oocytes [28]. The consequential effect is reduced or failed fertilization [29] and this appears to be related to the degree of response to gonadotrophin stimulation. A number of zona problems can be encountered leading to reduced or absent sperm binding [30,31] and these can be resolved by ICSI [23].

2.1.3. Unexplained infertility

Whilst large RCT studies indicate that unexplained infertility is not, by itself an indication for ICSI, the outcomes of any IVF application may reveal a relevant "field trial" [32]. Reduced fertilisation rates < 50% of mature oocytes in either an IVF-all [33] or an IVF-ICSI Split "trial" indicates a need to apply ICSI for future IVF-related procedures [13]. The idea of applying an IVF-ICSI Split approach as a diagnostic exercise for all first-up cases of unexplained infertility has been demonstrated to be a cost-effective approach in the long term [34].

2.1.4. Intrauterine insemination failures

Cases who had failed to achieve a biochemical pregnancy following 2–6 cycles of intrauterine insemination (IUI) were advised to consider ICSI or at least IVF-ICSI Split from our internal studies [35] and indicated by others [36,37]. From internal studies the fertilization rate of cases proceeding to IVF from failed IUI was significantly lower than those directly utilizing ICSI (49% vs 69%; $p < 0.001$), and occurrences of complete fertilisation failure were significantly higher (13.4% vs 2.9%; $p < 0.001$) causing a change in policy to recommend ICSI in such cases.

2.1.5. Genetic analysis of embryos

Where preimplantation diagnosis (PGD) and screening (PGS) was applied, the current recommendations are to utilize ICSI in order to avoid contamination of the embryo biopsy specimens (either blastomeres or trophoblast specimens) from sperm adherent to the zona

pellucida. This is recommended by the ESHRE PGD Consortium [38].

2.1.6. Cryopreserved gametes

Both cryopreserved spermatozoa as well as cryopreserved oocytes may show diminished fertilization capacity; in the former because of effects on the acrosomal cap and the latter mainly by effects on the zona pellucida. ICSI was advised when utilizing such cryopreserved gametes, especially when the slow-freeze technique was applied [24].

2.2. Indications for IVF only

Following our earlier study on IVF-ICSI Split [13], we have reserved the use of IVF Only on cases which exclude male factor infertility, and where satisfactory fertilization has been previously demonstrated in the IVF program or following an IVF-ICSI Split study. Such cases have shown a normal semen profile, as well as a low DNA fragmentation index (< 15%), along with > 4 oocytes recovered from the woman. Nonetheless, some severe male factor couples requested IVF Only or IVF-ICSI Split to determine if any IVF embryos could be created.

2.3. Study cohort

Fig. 1 outlines the breakdown of all IVF-related treatment cycles initiated across the period January 2014 to October 2017. This embraced a period of clinical and laboratory stability with conformity of practice among clinicians based on the PIVET Algorithms, mostly blastocyst culture of embryos, total vitrification method for cryopreservation and single embryo transfer (SET) policy for the vast majority with no cases exceeding the double embryo transfer (DET) for the selective remainder. From 1935 cycles initiated, 1547 were investigated further as the excluded cycles did not have full clinical and embryological outcomes and were either cancelled (n = 222), resulted in a failed OPU (n = 65), involved unfertilized oocyte freeze (n = 26), or gamete intrafallopian transfer (n = 5) cycles. Donors were excluded to minimize the influence of multiple confounders within single cases (n = 70). In this study with 1547 fertilization attempts, 1229 resulted in the transfer of a fresh embryo inseminated by IVF Only (n = 29), ICSI Only (N = 1067) or IVF-ICSI Split (N = 133). Freeze all embryo cycles occurred in 246 cases with insemination by IVF Only (n = 4), ICSI Only (N = 223) or IVF-ICSI Split (N = 19). Finally, the remaining 72 cases with a fertilisation attempt were excluded from analysis due to failed fertilization following insemination by IVF Only (n = 7), ICSI Only (N = 65) or IVF-ICSI Split (N = 0).

The outcome of those successful fertilization procedures was documented from embryo generation through fresh and frozen embryo transfers (FET), and to resultant clinical pregnancy determination as far as October 2017 (viable intra-uterine gestation detected by trans-vaginal ultrasound at 7 weeks) and live births (January 2014 to December 2016).

The IVF-ICSI Split group was analysed separately (Fig. 2) with respect to pregnancy and live birth outcome from either fresh transfers or FETs and the origin of the embryo transferred was sub-classified with respect to being an IVF-generated or ICSI-generated embryo. During the study period the clinic adopted an increasing commitment to SETs reaching 90% such being consistent with policies throughout Australia and New Zealand; ANZARD report [14].

2.4. Clinical protocols for IVF

The IVF-related protocols were stringently conducted according to rFSH dosage algorithms which also included the selection of stimulation protocol type (Flare, Antagonist & Long down-regulation) based on age and other physiological parameters (including body mass index; BMI, AFC and AMH level). Oocyte maturation trigger (agonist vs human chorionic gonadotrophin (HCG)) and luteal phase support was also managed by algorithms based on follicle numbers for the former and retrieved oocyte numbers for the latter. Oocyte recovery was performed using a single lumen 17 gauge needle where there were > 5 follicles and a 16 gauge double-lumen flushing needle if there were ≤5 follicles ≥14 mm diameter (COOK, Australasia). In keeping with the current ANZARD report [14], the vast majority of embryos were cultured to the blastocyst stage and women had a SET with residual blastocysts cryopreserved by Cryotop vitrification (Kitazato, Gytech, Australia). On the basis of repetitive failed ETs, a select few patients received a maximum of DET. When ≥20 oocytes were recovered, all embryos were cryopreserved although this rate was < 4.0% of OPUs [39]. With respect to the ICSI Only or IVF-ICSI Split insemination, the patients were advised of the clinical recommendation and the reasons for ICSI, and they made their final decision after taking into account the added out-of-pocket expense. Written informed consent was mandatory, for all processes including IVF Only, ICSI Only and IVF-ICSI Split insemination. Pregnancy rates and live birth rates were comparatively analysed with numerators as fresh, frozen or combined pregnancy or live birth instances and the denominator being cycle initiated, OPU or ET [40]. The combined fresh and frozen rates are referred to as pregnancy or live birth productivity rates.

2.5. Laboratory protocols

All oocyte-cumulus complexes (OCCs) were graded at recovery and distributed according to the designated insemination method (i.e. IVF Only, ICSI Only or IVF-ICSI Split). Oocytes were recovered into Hepes-buffered medium (Quinn's Advantage Sage; Origio Australasia) with flushing medium (Origio Flushing Medium; Origio Australasia) used when follicle flushing was undertaken. All oocytes were then placed in a bicarbonate-buffered resting medium (Quinn's Advantage Fertilization medium) for 3–4 h to allow completion of the M2 stage of meiosis prior to allocation for IVF or ICSI. The incubation conditions were within Minc solid-state incubators (COOK, Australia) at 37 °C gassed with humidified triple gas - 5% O_2, 6% CO_2 and 89% N_2.

Oocytes for IVF were maintained as OCCs and placed in Fertilisation medium (Quinn's Advantage) along with 100,000 spermatozoa prepared following centrifugation through a 2-layered colloidal silica suspension (Pure sperm 40/80; Nidacon, Sweden). The incubation conditions were under oil (Sage; Origio, Australia) which had been equilibrated overnight. The oocytes had pronuclear checks performed at 16–18 h post-overnight incubation and fertilization rates were reported as the number of two pronuclei zygotes (2 P N) per oocytes collected, allocated, and 2 P Ns per mature oocyte identified with polar body/bodies present. Oocytes for ICSI were prepared by immersion into Hyaluronidase solution (1500 IU Hyalase diluted in Quinn's Advantage Hepes for a final concentration of 80 IU/ml) for 30 s to disperse the cumulus cells and thereafter subjected to pipetting to partially strip the remaining cumulus and coronal cells [41,42]. This was performed within a humidified workstation chamber gassed with 6% CO_2 in air within Quinn's Fertilization medium. Only M2 (MII) oocytes were accepted for ICSI. The pronuclear check was performed at 16 h post-overnight incubation in Minc solid-state chambers. Fertilization was reported as 2 P Ns per oocytes collected, allocated and 2 P Ns per injected oocyte (i.e. per M2 oocyte). Oocytes at the germinal vesicle stage or persisting M1 stage were discarded along with those classified as degenerate, denuded or with a fractured zona. IVF Only insemination was essentially reserved for cases shown to have ≥50% fertilization rates on a previous IVF-ICSI Split study or where patients requested IVF-Only for theological, personal or cost-related issues.

All 2 P Ns were then placed into cleavage-stage medium (Quinn's Advantage Sequential medium) for culture to Day-3 at which stage ET was considered if there were fewer than 3 high-grade embryos progressing at the 6–8 cell stage. In most cases, there were at least 3 high-grade embryos, and these were transferred to blastocyst culture medium (Quinn's Advantage Sequential medium) for culture through to Day-5 or Day-6 when ET or cryopreservation by vitrification was

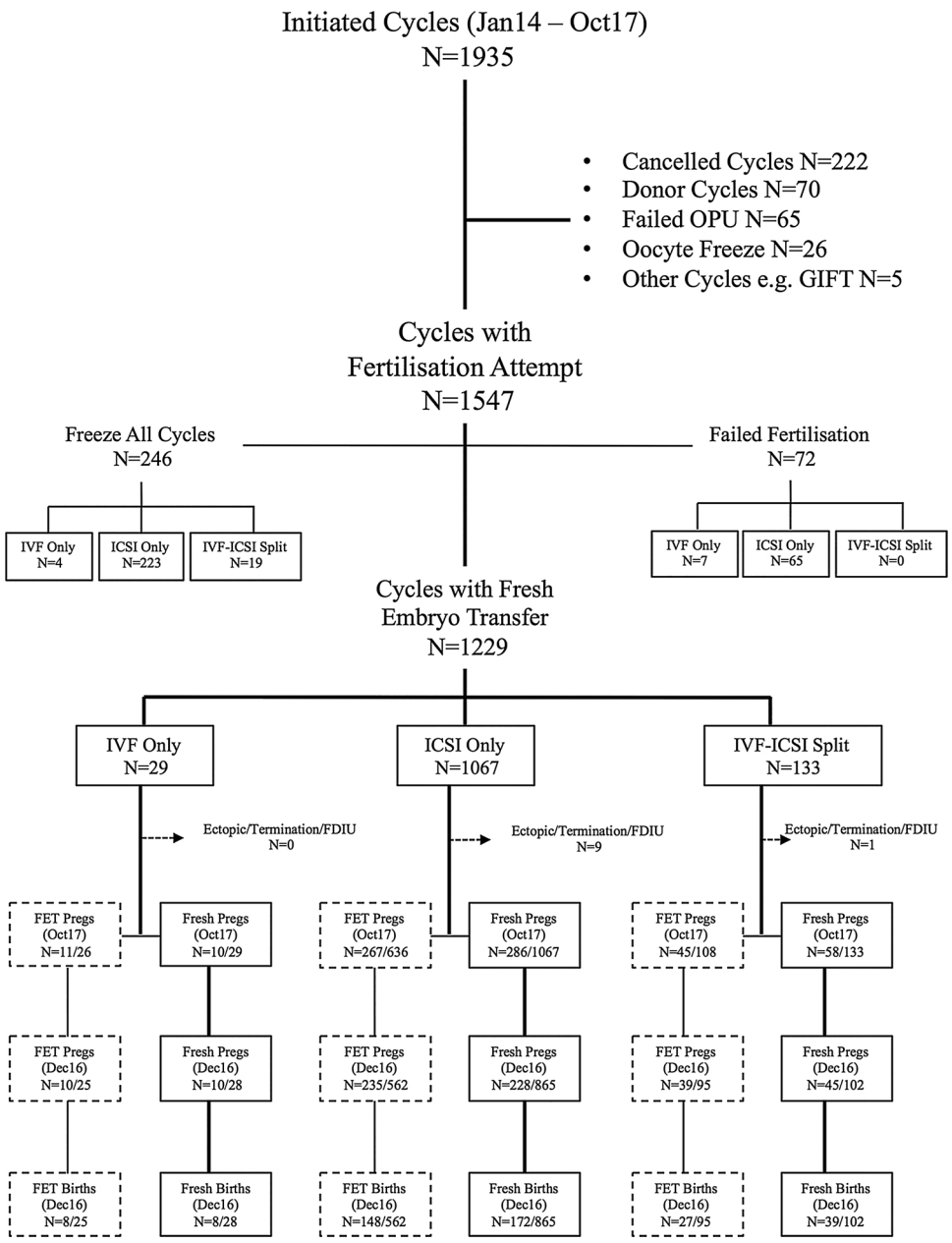

Fig. 1. Flowsheet depicting clinical outcomes from 1935 cART procedures utilising autologous gametes (eggs and sperm) in 3 modes – IVF Only, ICSI Only or IVF-ICSI Split inseminations. The treatment cycles and resultant pregnancies are based on data to October 2017. The livebirths are documented from cycles completed in December 2016 and delivered by October 2017.

considered. Embryos not at the blastocyst stage by Day-5, were left one more day to Day-6 and re-assessed. Any good quality blastocysts on Day-6 were then vitrified and the rest allowed to succumb.

2.6. Statistical aspects

The specific statistical methods included Chi-square for the main ratios and *t*-test of means and ANOVA for continuous normally distributed data. The confounders such as demographics, age, BMI, subcategories of semen analysis abnormalities, AFC and AMH categories were analysed by logistic regression in both univariate and multivariate models.

2.7. Ethical considerations

As this is a retrospective analysis of routine clinical practices conducted under licence from the RTC operating under legislation (Human Reproductive Technology Act of Western Australia 1991), specific ethical approval was not required. However, reporting of this data is approved by the Curtin University Human Ethics Committee under approval no. RD_25-10.

3. Results

Up to December 2016, 567 clinical pregnancies arose from a total 1677 fresh or frozen ETs (33.8%), with the distribution shown in Fig. 1. Consistent with the SET policy of the clinic, very few multiple pregnancies arose (10 from 219 fresh cycle live births). There were 9 sets of twins (4 from SET; 5 from DET) and one triplet (from a SET transfer) and there were no losses with all proceeding to live births of all infants although each instance was counted as a single live birth for statistical analysis. From the FET cycles, there were 2 sets of twins (1 from SET,

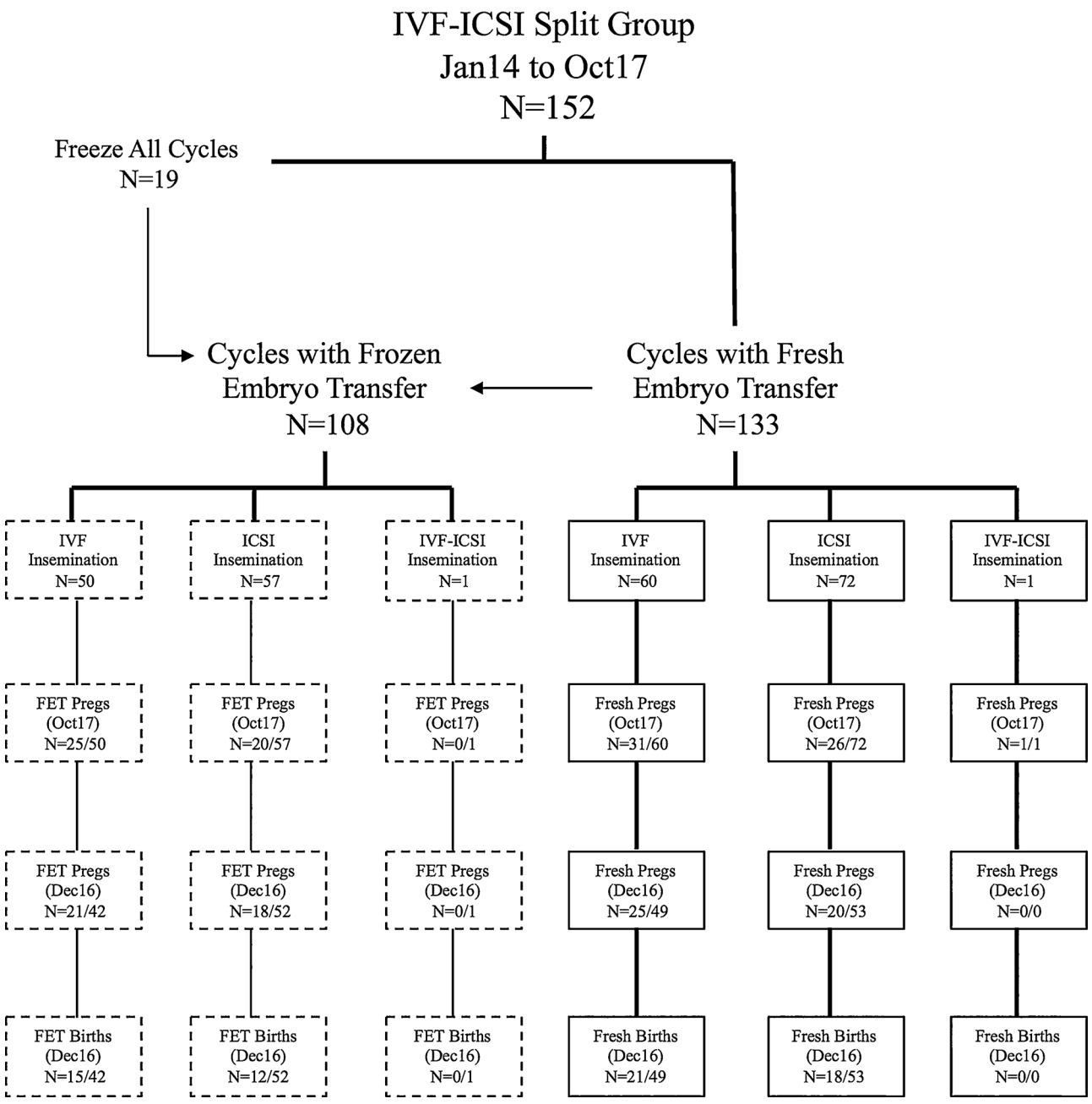

Fig. 2. Flowsheet depicting clinical outcomes from 152 ART procedures utilising autologous gametes in the IVF-ICSI Split mode where oocytes were randomly allocated for insemination. The treatment cycles and resultant pregnancies are based on data to October 2017. The livebirths are documented from cycles completed in December 2016 and delivered by October 2017. Includes two cases with double embryo transfer involving both an IVF- and ICSI-generated embryos. The Frozen transfer occurred in 2016, while the Fresh transfer occurred in 2017.

the other from DET) from 183 live births. Up to December 2016, the total number of live births in fresh and FET cycles was 402 (comprising 415 infants) with a multiple pregnancy rate per ET of 3.0%.

When the ART cycles were analysed according to the type of insemination, i.e. IVF Only, ICSI Only or IVF-ICSI Split, there were significant differences in the embryological outcomes (Table 1). The mean number of oocytes collected per OPU and the fertilisation rate represented by the mean number of 2 P Ns generated, was lowest in the ICSI Only group. However, the overall fertilisation rate per inseminated mature M2 oocyte was only significantly different between the ICSI Only (74.0%) and the IVF-ICSI Split group (79.7%) ($p = 0.033$). The IVF Only fertilisation rate (72.4%) was not significantly different from the ICSI Only or IVF-ICSI Split group. For oocytes receiving ICSI insemination, there was no significant difference in the fertilisation rate (mean number of 2 P Ns generated) per oocyte injected between the ICSI Only and IVF-ICSI Split groups, but a significant difference was observed when the rate was calculated per M2 oocyte injected with a higher rate shown in the IVF-ICSI Split group (81.9% versus 74.0%, $p < 0.000$). There was no significant difference in the fertilisation rate per IVF-inseminated oocyte or per inseminated M2 oocyte between the IVF Only and IVF-ICSI Split groups. However, examining the respective outcomes within the IVF-ICSI Split group showed a similar fertilisation rate for the IVF-inseminated M2 oocytes to the IVF Only group; but a significantly higher fertilisation rate for the ICSI-inseminated oocytes (81.9% vs 74.0%; $p < 0.000$). There were no significant differences between the insemination groups in relation to the quality of embryos generated or in the overall utilisation rates of oocytes or embryos.

When clinical outcomes were investigated, significantly more total

Table 1
Distribution of cases according to method of insemination category -IVF Only, ICSI Only or IVF-ICSI Split - showing the resulting embryological outcomes.

	Method of Insemination[A] (N = 1547)				
	IVF Only (N = 40)	ICSI Only (N = 1355)	IVF-ICSI Split (N = 152)	p-value	*Stats*
Number of Cycles with Fertilisation Attempt	40 (2.6%)	1355 (87.6%)	152 (9.8%)		
Cases with Male Factor, N (%)	18/40 (45.0%)	1001/1355 (73.9%)	112/152 (73.7%)	*< 0.000*	[1,2]
Oocytes Collected, Mean ± SD	11.8 ± 5.9	9.2 ± 5.9	13.9 ± 6.4	*< 0.000*	[1,3]
Mature M2 Oocytes Collected, Mean ± SD	9.1 ± 4.7	6.8 ± 4.6	10.7 ± 5.2	*< 0.000*	[1,3]
Total 2 P N Generated, Mean ± SD	6.8 ± 5.4	5.1 ± 3.9	8.4 ± 4.7	*< 0.000*	[1,3]
ICSI 2 P N, Mean ± SD	–	5.1 ± 3.9	4.8 ± 2.6	*0.205*	
IVF 2 P N, Mean ± SD	6.8 ± 5.4	–	3.6 ± 2.8	*< 0.000*	[2]
Oocytes Injected by ICSI, Mean ± SD	–	6.8 ± 4.6	5.9 ± 2.9	*0.001*	[3]
IVF Inseminated, Mean ± SD	11.8 ± 5.9	–	6.5 ± 3.1	*< 0.000*	[2]
FertRate (per oocyte collected), Mean % ± SD	57.0 ± 31.2	56.0 ± 24.6	60.6 ± 18.8	*0.087*	
FertRate (per M2 oocyte collected), Mean % ± SD	72.4 ± 38.5	74.0 ± 25.6	79.7 ± 25.2	*0.033*	[3]
ICSI FertRate (per M2 oocyte injected), Mean % ± SD	–	74.0 ± 25.6	81.9 ± 19.7	*< 0.000*	[3]
IVF FertRate (per M2 oocyte inseminated), Mean % ± SD	72.4 ± 38.5	–	69.6 ± 32.7	*0.523*	
High Quality Embryo Proportion, Mean % ± SD	10.8 ± 19.6	19.8 ± 26.6	18.0 ± 24.4	*0.119*	
Medium Quality Embryo Proportion, Mean % ± SD	63.0 ± 25.5	60.8 ± 29.3	63.3 ± 26.6	*0.548*	
Low Quality Embryo Proportion, Mean % ± SD	26.2 ± 21.2	19.4 ± 19.8	18.7 ± 14.8	*0.122*	
[B]Oocyte Utilisation Rate, Mean ± SD	26.9 ± 19.3	31.5 ± 22.5	31.1 ± 15.6	*0.408*	
[C]Embryo Utilisation Rate, Mean ± SD	48.7 ± 21.7	55.5 ± 30.8	52.9 ± 22.6	*0.290*	
Embryo Transferred Fresh, Mean ± SD	1.1 ± 0.3	1.2 ± 0.4	1.0 ± 0.2	*< 0.000*	[3]
Embryos Cryopreserved, Mean ± SD	2.4 ± 2.3	1.5 ± 2.1	3.4 ± 3.1	*< 0.000*	[1,3]

[A] Excluding: Cancelled cycles, Failed TVOA, Donor, Other (GIFT), and Oocyte Freeze.
[B] Total embryos transferred + total cryopreserved ÷ total oocytes collected.
[C] Total embryos transferred + total cryopreserved ÷ total 2 P N generated.
[1] IVF values significantly different from ICSI values (< 0.05).
[2] IVF values significantly different from Split values (< 0.05).
[3] ICSI values significantly different from Split values (< 0.05).

Table 2
Distribution of cases according to method of insemination category - IVF Only, ICSI Only or IVF-ICSI Split with resulting pregnancy and live birth outcomes.

	Method of Insemination[A] (N = 1547)			
	IVF Only (N = 40)	ICSI Only (N = 1355)	IVF-ICSI Split (N = 152)	p-value[B]
Failed Ferts, N (%)	7/40 (17.5%)	65/1355 (4.8%)	0/152 (0.0%)	*< 0.000*
Freeze ALL, N (%)	4/40 (10.0%)	223/1355 (16.5%)	19/152 (12.5%)	*0.263*
Ectopic/FDIU/Termination, N (%)	0 (0.0%)	9/1355 (0.7%)	1/152 (0.7%)	*0.869*
Pregnancy Outcomes (to October 2017)				
[C]Number of Cycles with Fresh Embryo Transfer	29	1067	133	
Fresh Pregnancies (Jan14-Oct17), N (%)	10/29 (34.5%)	286/1067 (26.8%)	58/133 (43.6%)	*< 0.000*
Number of Cycles with Frozen Embryo Transfer	26	636	108	
Frozen Pregnancies (Jan14-Oct17), N (%)	11/26 (42.3%)	267/636 (42.0%)	45/108 (41.7%)	*0.997*
Total Cycles with Embryo Transfer	55	1703	241	
Total Pregnancies (Jan14-Oct17), N (%)	21/55 (38.2%)	553/1703 (32.5%)	103/241 (42.7%)	*0.005*
Total pregnancies per OPU	21/40 (52.5%)	553/1355 (40.8%)	103/152 (67.8%)	
[D]*Live Births Outcomes (to December 2016)*				
Number of Cycles with Fresh Embryo Transfer[D]	28	865	102	
Fresh Births (Jan14-Dec16), N (%)	8/28 (28.6%)	172/865 (19.9%)	39/102 (38.2%)	*< 0.000*
Number of Cycles with Frozen Embryo Transfer[D]	25	562	95	
Frozen Births (Jan14-Dec16), N (%)	8/25 (32.0%)	148/562 (26.3%)	27/95 (28.4%)	*0.766*
Total Cycles with Embryo Transfer[D]	53	1427	197	
Total Births (Jan14-Dec16), N (%)	16/53 (30.2%)	320/1427 (22.4%)	66/197 (33.5%)	*0.002*
Total Livebirths per OPU	16/40 (40%)	320/1355 (23.6%)	66/152 43.4%)	

[A] Excluding: Cancelled cycles, Failed TVOA, Donor, Other (GIFT), and Oocyte Freeze.
[B] Statistical comparison using Chi square 3 × 2 Contingency Tables.
[C] Including: 10 cycles with Ectopic, Termination or FDIU.
[D] Restricted to cycles initiated before 31 Dec 2016.

fertilisation failure events occurred in the IVF Only group in comparison to the ICSI Only group (17.5% vs 4.8%; $p < 0.000$). However, there was not a single instance of failed fertilisation in the ICSI arm of the IVF-ICSI Split group where 152 fertilisation events were attempted (Fig. 1 & Table 2). Conversely, the IVF insemination arm had 18 fertilisation failures from 152 attempts. In addition, significantly more pregnancies and live births (43.6% and 38.2% respectively) were generated from the IVF-ICSI Split group when fresh embryos were transferred than from either the IVF Only or ICSI Only groups (Table 2; $p < 0.000$). The lowest rates were observed in the ICSI Only group (26.8% & 19.9%, respectively), while slightly higher rates were observed in the IVF Only group (34.5% & 28.6%, respectively). The data

for frozen embryos did not show any differences among the three insemination groups with respect to the chance of pregnancy or live birth per ET. Nonetheless when the fresh and frozen transfers were combined, the total pregnancies and total live births remained significantly higher for the IVF-ICSI Split group ($p < 0.005$ and $p < 0.002$, respectively). This was reflected in the live birth productivity rate per OPU rising to the highest level in the IVF-ICSI Split group (43.2%; $p < 0.000$).

However, analysis of potential confounders among the entire group showed that female patients treated in the ICSI Only group were significantly older with lower AMH levels and AFC grades; indicating lower ovarian reserve (Supplementary Table S1). With respect to male factors, as expected significantly more couples with male factor infertility were treated within the ICSI Only and IVF-ICSI Split groups (73.9% and 73.7%, respectively; p. < 0.000) compared with 45.0% in the IVF Only group. Further analysis showed that the ICSI group included more severe male factor cases - males with more severe reductions of semen volume, total sperm number, total motile sperm and sperm with progressive motility as well as those cases with more severe levels of morphological defects (Supplementary Table S2). Of particular interest the most severe male factor cases (Grade D sperm) achieved fewer pregnancies and live births across the groups; by approximately 50% in the univariate and 60% in the multivariate analyses, after factoring for method of insemination along with female factors - age and AMH level (Table 3). However, method of insemination did not alter pregnancy or live birth chance in the multivariate model (Table 3).

The fresh and frozen data described above included the transfer of IVF-generated and ICSI-generated embryos (meaning those embryos resulting after IVF-insemination and ICSI-insemination, respectively) within the IVF-ICSI Split group. The study then focused on the clinical outcomes specifically in this group with regard to fresh (Table 4) or frozen (Table 5) ETs. The clinical pregnancy rate per ET for IVF-generated and ICSI-generated embryos transferred fresh was 51.7% and 36.1%, respectively, while the live birth rate per ET was 42.9% and 34.0%, respectively. Although, there was a trend for increased outcomes in the IVF group, the observation was not significant ($p = 0.104$ & $p = 0.236$, respectively). For the fresh clinical pregnancy data (analysed up to October 2017) and the live birth dataset (analysed up to December 2016), there were no significant differences in mean female parameters such as age, BMI or AMH level (Table 4). Consequently, when adjusting both the clinical pregnancy rate and live birth rate according to female age, BMI or AMH individually, or in a multivariate regression model, no significant differences in pregnancy or live birth outcomes were observed (Supplementary Table S3). Adjustment for male semen parameters such as volume, sperm number, motility and morphology in an independent and multivariate regression model also did not demonstrate significant differences in pregnancy or live birth outcomes (Supplementary Table S3).

These results were similarly analysed for the outcomes of frozen transfers with IVF-generated or ICSI-generated embryos within the IVF-ICSI Split group (Table 5). Again, there were no significant differences among the female parameters (mean age, BMI and AMH), and only a non-significant trend towards higher clinical pregnancy and live birth rates when IVF fertilised frozen embryos were transferred. Specifically, the clinical pregnancy rate for IVF-generated and ICSI-generated embryos transferred frozen was 50.0% and 34.6%, respectively, while the live birth rate was 35.7% and 23.1%, respectively (Table 5). Logistic regression was then applied to adjust for female clinical factors and male semen parameters; effectively excluding any influence from these and showing the method of insemination did not influence the clinical pregnancy and live birth outcomes observed (Supplementary Table S3). This data can be summarised as showing that whilst IVF-ICSI Split resulted in complete exclusion of the problem of complete failed fertilisation in 152 cases, the embryos, whether generated by IVF or by ICSI, had a similar chance of implantation for both pregnancies and live births.

Finally, an overview investigation was performed to determine the fate of all embryos generated within the IVF-ICSI Split group (Table 6). A lower number of oocytes were inseminated using ICSI (893) than IVF (991) in the IVF-ICSI Split group. However, significantly more 2 P Ns were generated (730 versus 546, respectively), while ICSI demonstrated an expected higher fertilisation rate per mature M2 oocyte inseminated (81.9% versus 69.6%, respectively; $p < 0.000$). On average, the same number of IVF-generated and ICSI-generated embryos were transferred in fresh cycles (being 1.0 in accordance with SET policy). While similar numbers of embryos overall were cryopreserved in IVF and ICSI insemination (216 versus 307), the mean number of ICSI-generated embryos generated and cryopreserved per collection cycle was significantly higher (4.8 vs 3.6; $p < 0.000$; 2.0 vs 1.4; $p < 0.003$, per OPU respectively). Overall, 277 embryos from 546 IVF-fertilised embryos were either transferred fresh or cryopreserved (1.8 embryos per cycle), compared with 381 embryos from 730 ICSI-fertilised embryos were either transferred fresh or cryopreserved (2.5 embryos per cycle). While this appears significant, a non-significant utilisation rate per

Table 3
Logistic regression analysis of the entire study group to factor potential confounders of female clinical factors and male infertility factors (semen and sperm DNA fragmentation; SDF) impacting upon clinical pregnancies and live births.

	Whole Group - Clinical Pregnancy Chance (Oct17)				Whole Group - Live Birth Chance (Dec16)			
	Univariate (unadjusted)	p-value	Multivariate	p-value	Univariate (unadjusted)	p-value	Multivariate	p-value
Method of Insemination								
IVF-ICSI Split	1.00	–	1.00	–	1.00	–	1.00	–
IVF Only	0.68 (0.29–1.57)	0.369	0.75 (0.25–2.24)	0.612	0.65 (0.26–1.61)	0.348	0.78 (0.23–2.7)	0.701
ICSI Only	0.47 (0.33–0.68)	0.000	0.69 (0.43–1.09)	0.109	0.40 (0.26–0.62)	0.000	0.81 (0.49–1.35)	0.415
Female Age	0.90 (0.87–0.92)	0.000	0.88 (0.85–0.91)	0.000	0.89 (0.87–0.92)	0.000	0.87 (0.84–0.91)	0.000
Serum AMH	1.02 (1.01–1.02)	0.000	1.01 (1.00–1.01)	0.117	1.02 (1.01–1.03)	0.000	1.01 (1.00–1.02)	0.068
Female BMI	1.00 (0.97–1.02)	0.825	–	–	0.98 (0.95–1.01)	0.279	–	–
Male Infertility								
Male Factor No	1.00	–	1.00	–	1.00	–	1.00	–
Male Factor Yes	1.48 (1.07–2.04)	0.017	1.72 (1.15–2.58)	0.008	1.45 (1.00–2.11)	0.05	1.31 (0.83-2.06)	0.242
Semen Volume	1.03 (0.96–1.11)	0.380	–	–	0.99 (0.90–1.08)	0.743	–	–
Total Sperm Number	1.00 (1.00–1.00)	0.421	–	–	1.00 (1.00–1.00)	0.816	–	–
Sperm Concentration	1.00 (1.00–1.00)	0.715	–	–	1.00 (1.00–1.00)	0.927	–	–
Total Sperm Motility	1.00 (1.00–1.00	0.688	–	–	1.00 (1.00–1.01)	0.710	–	–
Total Progressive Motility	1.00 (1.00–1.00)	0.465	–	–	1.00 (1.00–1.00)	0.878	–	–
Grade A Sperm	1.65 (0.83–3.29)	0.157	–	–	1.28 (0.55–2.99)	0.562	–	–
Grade D Sperm	0.53 (0.30–0.93)	0.028	0.45 (0.20–0.98)	0.044	0.48 (0.24–0.98)	0.045	0.32 (0.12-0.81)	0.044
Average SDF	0.99 (0.97–1.00)	0.141	–	–	0.99 (0.97–1.01)	0.294	–	–
% Normal Morphology	0.99 (0.96–1.02)	0.533	–	–	0.96 (0.92–1.00)	0.070	–	–

Table 4
Distribution of potential confounders in respective arms of IVF-ICSI Split group with identification of pregnancy and live birth rates resulting from fresh embryo transfers.

	IVF-ICSI Split Group Only (N = 151)[A]				
	No Transfer (Freeze All N = 19)	Fresh-IVF ET (N = 60)	Fresh-ICSI ET (N = 72)	p-value	*Stats*
Failed Ferts, N (%)	0/19 (0.0%)	0/60 (0.0%)	0/72 (0.0%)	–	
Freeze ALL, N (%)	19/19 (100.0%)	0/60 (0.0%)	0/72 (0.0%)	–	
Ectopic/FDIU/Termination, N (%)	0 (0.0%)	1/60 (1.7%)	0/72 (0.0%)	–	
Age in Years, Mean ± SD	33.2 ± 4.2	32.2 ± 4.4	33.3 ± 4.4	*0.546*	
BMI kg/m^2, Mean ± SD	25.2 ± 5.6	22.8 ± 4.8	23.8 ± 4	*0.112*	
AMH pmol/L, Mean ± SD	44.4 ± 38.4	32.2 ± 29.8	28.5 ± 20.9	*0.222*	
Pregnancy Outcomes (to October 2017)					
Number of Cycles with Fresh Embryo Transfer	–	60	72	–	
Fresh Pregnancies (Jan14-Oct17), N (%)	–	31/60 (51.7%)	26/72 (36.1%)	*0.104*	
Age in Years, Mean ± SD	–	32.6 ± 4.4	32.6 ± 4.2	*0.994*	
BMI kg/m^2, Mean ± SD	–	23.7 ± 4.3	22.4 ± 5.0	*0.161*	
AMH pmol/L, Mean ± SD	–	30.1 ± 19.4	32.1 ± 29.9	*0.687*	
Live Birth Outcomes (to December 2016)					
[B]Number of Cycles with Fresh Embryo Transfer	–	49	53	–	
Fresh Births (Jan14-Dec16), N (%)	–	21/49 (42.9%)	18/53 (34.0%)	*0.236*	

[1]IVF values significantly different from ICSI values (< 0.05).
[2]IVF values significantly different from No ET values (< 0.05).
[3]ICSI values significantly different from No ET values (< 0.05).
[A] Excluding: Cancelled cycles, Failed TVOA, Donor, Other (GIFT) and Oocyte Freeze. 1 cycle with the Fresh Transfer of an IVF and an ICSI Embryo resulting in a Clinical Pregnancy was excluded from this Table (N = 152).
[B] Restricted to cycles initiated before 31 Dec 2016.

Table 5
Distribution of potential confounders in respective arms of IVF-ICSI Split group with identification of pregnancy and live birth rates resulting from frozen embryo transfers.

	IVF-ICSI Split FETs Only (N = 107)[A]		
	IVF FET (N = 50)	ICSI FET (N = 57)	p-value
Age in Years, Mean ± SD	33.2 ± 4.1	32 ± 3.5	0.227
AMH pmol/L, Mean ± SD	39.2 ± 29.8	28.9 ± 20.6	0.069
BMI kg/m^2, Mean ± SD	24.3 ± 4.4	23.5 ± 4.4	0.493
Pregnancy & Birth Outcomes			
FET Pregnancies (Jan14-Oct17), N (%)	25/50 (50.0%)	20/57 (35.1%)	0.169
Number of FET Cycles (Jan14-Dec16)[B]	42	52	
FET Births (Jan14-Dec16), N (%)	15/42 (35.7%)	12/52 (23.1%)	0.329

[A] Excluding: Cancelled cycles, Failed TVOA, Donor, Other (GIFT) and Oocyte Freeze. 1 cycle with the Transfer of an IVF and an ICSI Frozen Embryo that did not result in a Clinical Pregnancy was excluded from this Table (N = 108).
[B] Restricted to cycles initiated before 31 Dec 2016.

2 P N-generated was observed for the IVF and ICSI groups (50.7% versus 52.2%) during the study period; although there remain more ICSI-generated embryos in cryostorage for future potential ETs.

With respect to fetal outcomes, pregnancy losses were different among IVF and ICSI generated pregnancies. The IVF Only group lost 4 pregnancies (4/20; 20.0%), the ICSI Only group lost 143 pregnancies (143/463; 30.9%) and the IVF-ICSI Split group lost 18 (18/84; 21.4%). The ICSI group showed significantly higher losses than the IVF-ICSI Split group ($p < 0.001$) but not in comparison to the IVF Only group (perhaps from small case number in the latter). When the IVF-ICSI Split group was sub-analysed, 10 pregnancies were lost from the IVF-generated arm (10/46; 21.7%) and 8 from the ICSI-generated arm (8/38; 21.1%). There were no significant differences. There were 8 significant congenital abnormalities among the 415 infants (1.9%). These were all from ICSI-derived embryos, bearing in mind that 90% of all pregnancies were ICSI inseminated. Two of the abnormalities were Trisomy 21 pregnancies, one terminated at 22 weeks, the other proceeding to term with no other detectable health issues. Two other pregnancies with neural defects (meningocele and spina bifida) resulted in spontaneous stillbirths at 20 weeks and 22 weeks respectively. Congenital abnormalities in the 5 live births ranged across umbilical hernia, two cases of diaphragmatic hernia and one of testicular torsion requiring surgical removal.

4. Discussion

Having commenced IVF prior to the first birth in 1978 (JLY 41 yrs), members of our group recognised the limitations in managing infertility by this method especially when there are recognisable disorders in the semen profile [43]. Initially, achieving pregnancies with so-called male factor infertility were quite limited, improving dramatically after ICSI was developed [1]. The majority of cases in our study where ICSI Only was applied, were for male factor (73.9%) being similar to other recent reports [44]. Many other fertility factors also proved to be better managed by ICSI and this retrospective study attempts to examine both the utility of extending the indications, as well as the safety given that the technique bypasses many natural selection processes.

Since maternal age, BMI, AMH levels, and AFC ratings were used to decide on method of fertilization, ICSI was more commonly performed among women of advanced age, women with higher BMI, and women with lower AMH levels as well as AFC ratings. This study shows that ICSI was used exclusively on 86.8% of cases whilst IVF Only was used on 2.4%, the remaining 10.6% having an IVF-ICSI Split procedure. In the total cohort, fertilisation rates and utilisation rates showed differences favouring IVF, and there were apparently more pregnancies and live births following IVF, which was significant for fresh transfers. However, these comparisons are not optimal for statistical evaluation given the distribution of widely different underlying clinical and laboratory factors predicating the allocation of oocytes. Apart from that issue, analysis of the confounders indicated that ICSI was performed in significantly more cases of women with advanced age (≥ 40 years), and with significantly higher BMI levels, particularly in the obese range, as well as having significantly lower AMH levels and lower AFC ratings.

Table 6
Distribution of cases according to method of insemination of randomised oocytes - IVF or ICSI - within the IVF-ICSI Split category showing the resulting embryological outcomes.

	IVF-ICSI Split Group Only (N = 151)[A]				
	IVF Fertilised	ICSI Fertilised	Total	p-value	*Stats*
Number of Cycles with Oocyte Collection, N	151	151	–		
Total Oocytes Retrieved, N	–	–	2119		
Total Split Oocytes with Fertilisation Attempt, N	991	893	1884		
Total M2 Oocytes with Fertilisation Attempt, N	785	893	1678		
Mean Oocytes with Attempted Fertilisation per Collection Cycle	6.6	5.9	–		
Mean M2 Oocytes with Attempted Fertilisation per Collection Cycle	5.2	5.9	–		
Total 2 P N Zygotes Generated, N	546	730	1276	*< 0.000*	[B]
2 P N Zygotes Generated per Collection Cycle, Mean ± SD	3.6 ± 2.8	4.8 ± 2.6	–	*< 0.000*	[t]
FertRate per Oocyte with Fertilisation Attempt (%)	55.1	81.9	–		
FertRate per M2 Oocyte with Fertilisation Attempt (%)	69.6	81.9	–		
Number of Fresh Embryo Transfer Cycles, N	60	72	132		
Total Split Embryos Transferred in Fresh Cycles, N	61	74	135[A]		
Mean Embryos Transferred per Fresh Transfer Cycle	1.0	1.0	–		
Total Split Embryos Cryopreserved, N	216	307	523	*0.470*	[B]
Embryos Cryopreserved per Oocyte Collection Cycle, Mean ± SD	1.4	2.0	–	*0.003*	[t]
Total Embryos Transferred Fresh or Cryopreserved, N	277	381	658	*< 0.000*	[B]
Mean Embryos Transferred Fresh or Cryopreserved per Oocyte Collection, N	1.8	2.5	–		
Overall Embryo Utilisation per 2 P N Generated (%)	50.7	52.2	–		

[A] Total of 137 Fresh Embryos were Transferred in the Entire Split Group, 1 Cycle was excluded here which involved the Fresh Transfer of 1 IVF and 1 ICSI Embryos in combination (N = 152).
[B] Chi square 2 × 2 contingency table.
[t] T-test.

All of this reflects the evolution of allocation of cases to ICSI Only, where early problems in patients categorised as poor prognosis were apparently improved by applying the ICSI method for more assured fertilisation. Whilst the modern era of evidence-based medicine demands more rigorous methodology for studying treatment protocols, long–standing IVF clinics will generally be reluctant to revert to reviewing their case allocation by re-applying randomised controlled studies (RCTs) to determine the best approach. Their reluctance will relate to the identification of numerous variables recognised as confounders in IVF treatments, perhaps well over 100 apart from the usual suspects (female age, BMI, AFC, AMH, smoking) and could include dietary and occupational effects as well as exposure to toxins, poor air quality and sedentary behaviour. Emotional stresses, anxiety levels and depression can be treatment-related as well as inherent from non-fertility causes. However, all may well confound results and outcomes in IVF [45].

Of course, laboratory issues can also vary and influence outcomes; one can only hope that large data collections and adherence to optimised conditions can minimise such effects (Vienna consensus; [46]). Of interest, the Vienna consensus group does not comment on ICSI allocation rates, supporting our view that the appropriate rate is a discretionary matter for individual clinics depending upon clinical case-mix and clinic policies. Such is consistent with advisory bodies who encourage the application of ICSI to maximise the chances of pregnancy whilst advising against universal use without indication (ASRM; [47]). Whilst male-factor remains the main indication for ICSI, its application outside this has been rising [48] despite uncertain evidence [49].

It is probably only in the IVF-ICSI Split group where a reasonable comparison of outcomes can be analysed bearing in mind this group comprises mainly the unexplained or poorly explained factors including some of the milder male-factor cases. Thus, this composition may conceal a wide range of poorly understood infertility factors that may include metabolic, genetic and epigenetic aberrations. Our experience indicates that many couples prefer the idea of IVF-ICSI Split on the basis that if they achieve IVF-inseminated embryos such are preferred as being more natural; but the ICSI –inseminated embryos protect against the emotional distress of complete failed fertilisation and provides a back-up situation should the IVF-generated embryos fail to implant. Our favourable experience with the IVF-ICSI Split is matched from other studies showing prevention of fertilization failure [50,51] and the generation of higher numbers of good quality embryos [52–54].

Within the entire cohort, both pregnancy and live births rates appeared higher in the IVF and IVF-ICSI Split groups, but when the confounders were adjusted, there were no significant differences. It appears that pregnancies arising from both IVF-generated and ICSI-generated embryos had a similar chance of becoming live births and no significant abnormalities were recorded from either. Nonetheless the extended data arising from the IVF-ICSI Split group showed a higher number of embryos were generated from the ICSI arm and the embryo utilisation number was also higher from the ICSI-generated embryos. However, neither the chance of pregnancy nor the likelihood of live birth was any different between these groups, although there remain more blastocysts in cryostorage awaiting ET procedures from the ICSI-generated group. Such blastocysts can be expected to have equivalent implantation potential to IVF-generated embryos as reflected by the frozen outcomes in the current study, and as identified elsewhere [10]. Furthermore, this translates into a higher productivity rate of around 60% per OPU for the patients managed within the IVF-ICSI Split group, in part because of the higher fertilisation outcomes. This is fully consistent with earlier reports [9,55] where the only reason advising caution about ICSI related to the higher fetal abnormality rates which had been reported.

With respect to the significant finding of higher pregnancy wastage in the ICSI Only group when compared to the IVF-ICSI Split, this needs to be interpreted against the finding of no significant difference compared to the IVF Only group, albeit a small sample size, and no significant differences in pregnancy losses between pregnancies derived from IVF-generated embryos and those from ICSI-generated embryos. These findings all point to the allocation of oocytes into the various strategic programmes – IVF Only for normal, young couples; ICSI for male factor and adverse female factors; and IVF-ICSI Split for unexplained or poorly explained infertility. Resultant embryo quality does not seem to be influenced by the method of fertilisation whether IVF or ICSI, and more likely depends upon intrinsic factors within the gametes [56]. This means the embryos derived from each group in our study will carry the sequelae of the underlying factors, regardless of the method of insemination.

With respect to fetal abnormalities, historically the early data showed IVF carried significant risk compared to the naturally conceived

children; however, following the wider use of ICSI, such difference has disappeared, taken up now by the ICSI conceived children (7.1% vs 4.0%) [6]. In fact if the underlying male factor population is carefully analysed and the data is adjusted for confounders, it appears there is no increase in abnormalities from the ICSI technique but the elevated rate of abnormalities in offspring is attributed to the genetically-associated problems underlying the infertility of the male or female from whom the gametes were derived [8]. Such paternal effects operating during ICSI and affecting embryo development were described by Mendoza's group [57], but maternal influences will also operate such as female age and low AMH reflecting depleted ovarian reserve, as shown in our study. The current data indicated a low 1.9% rate for congenital abnormalities among the 415 infants where almost 90% were ICSI-derived supports the view that there is no major cause for concern regarding any IVF-related procedures in the current era. This improved outlook is undoubtedly reflective of higher quality laboratory practices including the vitrification technique for cryopreservation as espoused in the Vienna consensus [46].

We acknowledge that the data presented in this study is subject to various limitations including being retrospective in nature and does not represent an analytical appraisal of ICSI versus IVF outcomes, which would require a prospective RCT. Such a study would enable appropriate matching of clinical details and groupings with similar numbers, being more relevant for statistical analyses. The present study is an audit of current practice which has evolved following the experience of clinical limitations, such as unexpected complete failed fertilization. Nonetheless, the findings from this audit indicate practices which favour our selection system for ICSI. Furthermore, the observations from the IVF-ICSI Split group may be distinctly relevant as they are derived from more closely matched sample sizes and involved randomisation of oocytes to each insemination mode. However, since the data analysis is retrospective, the interpretation of the findings must be considered with caution.

We would conclude that IVF clinics should offer patients strategies designed to maximise the number of embryos generated from the individual woman's pool of oocytes recovered. For first-up couples, this should be ICSI for the conditions described in the methodology of this study and IVF-ICSI Split for those without clear indications for ICSI. We support the view that IVF and ICSI are complementary techniques in the management of unexplained and poorly explained infertility [56–58] as there arises the opportunity for utilising the embryos derived from either or both techniques enabling wider choice opportunities for the patient. IVF Only should ideally be reserved for those cases with proven normal fertilisation on a previous IVF-ICSI Split protocol which can be considered to have both diagnostic and therapeutic benefits [57]. ICSI can generate more embryos than IVF, and such embryos appear to have a similar chance of implantation without any apparent elevation of fetal abnormalities. Therefore, ART clinics should utilize ICSI according to their own discretion based on the case-mix of their patients.

Author's contributions

JLY and JLC conceived the idea of this study following a challenge from the Reproductive Technology Committee which is the authoritative body that regulates ART in Western Australia. Data was extracted from the PIVET database managed by PMH and laboratory data deficiencies were corrected by NM. Data analyses including logistic regression analyses were conducted by SSD, KNK and YY. Laboratory aspects were reviewed by JLC and NM. The manuscript was prepared and the study supervised by JLY and KNK equally.

Conflicts of interest

There are no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.repbio.2018.05.002.

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