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Randomized allocation of oocytes to IVF or ICSI for IVF-naïve cases with unexplained infertility in an IVF-ICSI Split protocol favors ICSI to optimize live birth outcomes

John Lui Yovich [1, 2, *], Jason Lee Conceicao [1], Nicole Marjanovich [1], Rachel Wicks [1], Jesmine Wong [1] and Peter Michael Hinchliffe [1]

[1] *PIVET Medical Centre Perth, Western Australia, Australia 6007.*
[2] *Department of Pharmacy and Biomedical Sciences, Curtin University Perth, Western Australia, Australia 6845.*

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Abstract

In assisted reproduction treatments (ART), applying the ICSI method for fertilization of oocytes rather than traditional IVF method, is regarded as controversial for two reasons, namely utility and safety. Our study examines an IVF-ICSI Split model for couples with unexplained infertility, where male factor is meticulously excluded and ART is conducted by a strict algorithm, a commitment to blastocyst culture, along with single embryo transfers and a high commitment to cryopreservation. From 242 treatment cycles, 3346 oocytes recovered (13.8 per OPU) were randomly allocated to IVF or ICSI and the fertilization rates standardized to the number of 2PNS arising from each group applying the metaphase II oocyte number identified for the ICSI group, as the denominator for both groups. The fertilization rates were significantly higher overall for ICSI (83.2% vs 65.4%; p<0.0001), being most pronounced for women under 40 years. The resultant embryos had equivalent implantation rates in both fresh ET and frozen (FET) cycles with no significant differences in pregnancy rates, miscarriage rates or live birth outcomes indicating equivalent embryo quality. However, there were significantly higher numbers of ICSI-generated embryos cryopreserved and subsequent FET procedures showed higher live birth rates (21 births vs 6 births; p<0.005) and potential livebirths (214 births vs 104 births; p<0.0001). No congenital fetal abnormalities were detected in any of the 199 babies delivered during the study period to December 2020, neither IVF-generated nor ICSI-generated. Whilst the data strongly favors ICSI, there were 2 women (from 26 with fertilization in one arm only) who demonstrated fertilization only in the IVF arm of the study. We conclude that the IVF-ICSI Split model should be undertaken on all IVF-naïve women with unexplained infertility to determine the appropriate fertilization mode, albeit ICSI will be safely preferred for >90% of cases.

Keywords: Assisted reproduction treatments (ART); *In vitro* fertilization [IVF]; Intracytoplasmic sperm injection [ICSI]; Single embryo transfer (SET); Frozen embryo transfer (FET); Live birth productivity rate (LBPR)

1. Introduction

In vitro fertilization (IVF) has become a popular treatment mode for infertility due to female factors since it first success in 1978 [1-8]. With respect to infertility related to male factors, modified sperm preparation techniques were introduced from 1984 [9-11], including the addition of in-vitro adjuvant pentoxifylline [12-14], as well as micromanipulation procedures such as partial zona dissection (PZD) [15], zona drilling and later, sub-zonal insemination (SUZI) [16,17], similarly micro-insemination sperm transfer (MIST) [18] with and without pentoxifylline [17]. Finally, all the previous methods were surpassed by the introduction of intra cytoplasmic sperm injection ICSI [19] in 1992 which enabled the application of IVF to an even wider range of cases, including the use of epididymal, thereafter testicular, sperm [20] and the outcomes can benefit by the combination of pentoxifylline to overcome motility

* Corresponding author: John L Yovich
PIVET Medical Centre Perth, Western Australia Australia 6007.

deficiencies in the sperm preparations prior to sperm injection [21,22]. Currently, it is estimated more than 10 million infants have been derived from the IVF±ICSI methodologies.

However, these historical advances in managing infertility caused some trepidation as numerous physiological processes have become sequentially bypassed. These include the molecular passport processes [23,24] underlying the selection of those approximately 20 spermatozoa which reach the oocyte-cumulus complex (OCC) within the fallopian tube for natural conception [25,26]. Even at the OCC site, several complex natural processes are bypassed when ICSI is utilized. These include hyperactivated motility [27], the acrosome reaction [28], cumulus dispersal [29], sperm-zona binding and sperm penetration followed by sperm-oolemma binding [30-31]. These processes ensue in response to oocyte-sperm activation, the molecular events being well summarized recently [32,33]. Although these processes are all by-passed by the ICSI technique, does it matter to the main desire, that of generating children for infertile couples? The subsequent processes leading to zygote formation, are completed by sperm-oocyte activation which still occurs following ICSI. There are 2 main molecular contenders for oocyte activation, namely phospholipase C-zeta and post acrosomal-WWW-P binding domain protein (PAWP) [34]. Furthermore, studies on non-human primates may also be of concern, revealing ICSI may potentially affect pronuclear orientation, cleavage patterns, and microtubule configurations (which influence zygote polarity and embryonic axes) [35-37].

Currently, there is debate around the use of ICSI for non-male factor infertility (so-called unexplained infertility) whereby the proportion of ICSI cases may rise above the standard of around 50% [38] to levels of 80-100%. PIVET currently applies an ICSI rate ~90% justified by the demonstration of a higher number of live births. The PIVET protocols include the idea of an IVF-ICSI Split protocol for all IVF-naïve cases with unexplained infertility to avoid cases of complete fertilization failure [39,40]. Furthermore, it can define the best approach for future IVF treatments, particularly where reduced rates of fertilization may occur in one or other arm of the protocol [41-42]. A higher number of live births has recently been shown from this 3-arm policy where IVF-ICSI Split is applied for IVF-naïve cases presenting with unexplained infertility and either IVF or ICSI is undertaken in future cycles accordingly [43]. This study examines the outcomes from the IVF-ICSI Split arm over the past decade.

2. Material and methods

The data presented here is an observational study covering the most recent 10-year period of activity (2011-2020) in an ART facility which has an historical pioneer connection with the earliest forays into human IVF [3,4]. The data has been collected into the internal validated data base in real-time and analyzed retrospectively.

PIVET was established in 1981 following team leader JLY returning from his 4-year study period in London establishing an IVF facility with Professor Ian Craft across the period 1976-1980 [3-5]. Pregnancies from IVF were established in the latter half of 1981 and the first live birth occurred in July 1982 [44]. The insemination procedures were conducted by standard IVF protocols, and ICSI commenced in 1994 after a protracted period of deliberations by PIVET's Human Research Ethics Committee (HREC). ICSI was initially applied for clear cases of identifiable severe male factor, and later introduced for cases of poorly explained infertility. For this study, the following protocols have been applied:

2.1. ART Protocols at PIVET

PIVET's IVF procedures have been well documented over the 40 years of operation and standardized across this study period 2011-2019 [7,45]. The clinical management is undertaken according to defined FSH-dosing Algorithms, one of which is shown in Table 1 and was first published in 2012 [46] with validation of an optimized pregnancy productivity rate, and almost complete avoidance of ovarian hyperstimulation syndrome (OHSS) [47]. In almost 90% of OPU's oocyte numbers range from 8-12 for standard cases, adapted for a long-acting FSH product [48] and adjusted for lower numbers suited to a low-cost, minimal intervention program [49].

Table 1 One of PIVET's FSH-dosing Algorithms enabling increments of 12.5 IU: suited for Gonal-F or biosimilars across the range of 37.5-450 IU (red sector), Puregon across the range 125-450 IU (orange sector) and Elonva in the narrow range of 200-400 IU (green sector). A separate Algorithm for Puregon enables 8.3 IU increments in the range of 41.7-125 IU. Dosages are increased according to the woman's Age, diminishing AFCs and elevated BMI as well as her baseline FSH level, her smoking history, and the consideration of banking oocytes for self or donating. The Algorithm is presented as Table 1a for AFC Groups A, A+ and A++ with high AFC's >20 follicles; Table 1b for Groups B and C with mid-range AFCs 9-19 follicles; and Table 1c for Groups D and E with low AFCs ≤8 follicles. The legend for all tables is under Table 1c

Table 1a PIVET rFSH Dosing Chart suits Gonal-F & Biosimilars																
AMH		**>30 pmol/L**					**25-29.9 pmol/L**					**20-24.9 pmol/L**				
AFC*		**A++ (≥ 40 follicles)**					**A+ (30-39 follicles)**					**A (20-29 follicles)**				
BMI kg/m^2		16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35
Age (yrs)	20	37.5	37.5	7.5	50.0	50.0	50	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5
	21	37.5	37.5	37.5	50.0	50.0	50	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5
	22	37.5	50.0	50.0	50.0	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5	87.5	100.0
	23	50.0	50.0	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5	87.5	100.0	100.0	100.0
	24	50.0	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5	87.5	100.0	100.0	100.0	112.5
	25	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5	87.5	100.0	100.0	100.0	112.5	112.5
	26	62.5	62.5	62.5	75.0	75.0	75	87.5	87.5	87.5	100.0	100.0	100.0	112.5	112.5	112.5
	27	62.5	62.5	75.0	75.0	75.0	87.5	87.5	87.5	100.0	100.0	100.0	112.5	112.5	112.5	125.0
	28	62.5	75.0	75.0	75.0	87.5	87.5	87.5	100.0	100.0	100.0	112.5	112.5	112.5	125.0	125.0
	29	75.0	75.0	75.0	87.5	87.5	87.5	100.0	100.0	100.0	112.5	112.5	112.5	125.0	125.0	125.0
	30	75.0	75.0	75.0	87.5	87.5	100.0	100.0	100.0	112.5	112.5	112.5	125.0	125.0	125.0	137.5
	31	75.0	75.0	87.5	87.5	100.0	100.0	100.0	112.5	112.5	112.5	125.0	125.0	125.0	137.5	137.5
	32	87.5	87.5	87.5	100.0	100.0	100	112.5	112.5	112.5	125.0	125.0	125.0	137.5	137.5	137.5
	33	87.5	87.5	87.5	100.0	100.0	100	112.5	112.5	112.5	125.0	125.0	125.0	137.5	137.5	150.0
	34	87.5	87.5	100.0	100.0	112.5	113	112.5	112.5	125.0	125.0	125.0	137.5	137.5	150.0	150.0
	35	100.0	100.0	112.5	112.5	112.5	125.0	125.0	125.0	137.5	137.5	137.5	150.0	150.0	162.5	162.5
	36	100.0	100.0	112.5	112.5	125.0	125.0	125.0	137.5	137.5	137.5	150.0	150.0	162.5	162.5	175.0
	37	100.0	100.0	112.5	112.5	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5
	38	112.5	112.5	125.0	125.0	125.0	125	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5
	39	112.5	112.5	125.0	125.0	137.5	138	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5
	40	112.5	112.5	125.0	137.5	137.5	138	150.0	150.0	150.0	162.5	162.5	175.0	187.5	187.5	200.0
	41	125.0	125.0	137.5	137.5	150.0	150.0	150.0	162.5	162.5	162.5	175.0	187.5	187.5	200.0	200.0
	42	125.0	125.0	137.5	150.0	150.0	163	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	225.0
	43	125.0	137.5	150.0	150.0	162.5	163	175.0	175.0	187.5	187.5	200.0	212.5	212.5	237.5	250.0
	44	137.5	137.5	150.0	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	225.0	225.0	237.5	250.0
	45	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	225.0	225.0	250.0	250.0

Table 1b PIVET rFSH Dosing Chart suits Gonal-F, Puregon and Elonva											
AMH		**15-19.9 pmol/L**					**10-14.9 pmol/L**				
AFC*		**B (13-19 follicles)**					**C (9-12 follicles)**				
BMI kg/m²		16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35
Age (years)	20	100.0	100.0	100.0	112.5	112.5	125.0	125.0	137.5	137.7	137.7
	21	100.0	100.0	100.0	112.5	112.5	125.0	125.0	137.5	137.7	137.7
	22	100.0	100.0	112.5	112.5	112.5	125.0	125.0	137.5	137.5	150.0
	23	112.5	112.5	112.5	125.0	125.0	125.5	137.5	137.5	150.0	150.0
	24	112.5	112.5	125.0	125.0	125.0	137.5	137.5	150.0	150.0	162.5
	25	112.5	125.0	125.0	125.0	137.5	137.5	150.0	150.0	150.0	162.5
	26	125.0	125.0	125.0	137.5	137.5	137.5	150.0	150.0	162.5	162.5
	27	125.0	125.0	137.5	137.5	137.5	150.0	150.0	150.0	162.5	162.5
	28	125.0	137.5	137.5	137.5	150.0	150.0	150.0	162.5	162.5	175.0
	29	137.5	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5
	30	137.5	137.5	150.0	150.0	150.0	162.5	162.5	175.0	175.0	187.5
	31	137.5	150.0	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5
	32	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0
	33	150.0	150.0	162.5	162.5	175.0	187.5	200.0	200.0	212.5	212.5
	34	162.5	162.5	175.0	175.0	187.5	187.5	200.0	212.5	225.0	237.5
	35	175.0	175.0	175.0	187.5	200.0	200.0	212.5	225.0	237.5	250.0
	36	175.0	187.5	200.0	200.0	225.0	225.0	237.5	237.5	250.0	262.5
	37	187.5	200.0	212.5	212.5	225.0	237.5	250.0	262.5	275.0	287.5
	38	187.5	200.0	212.5	225.0	237.5	250.0	262.5	275.0	287.5	300.0
	39	200.0	212.5	225.0	237.5	250.0	275.0	287.5	300.0	312.5	325.0
	40	225.0	237.5	250.0	262.5	275.0	300.0	312.5	325.0	337.5	350.0
	41	225.0	250.0	262.5	275.0	287.5	300.0	325.0	350.0	375.0	400.0
	42	250.0	262.5	275.0	287.5	300.0	325.0	350.0	375.0	400.0	425.0
	43	262.5	275.0	287.5	300.0	312.5	350.0	375.0	400.0	425.0	450.0
	44	275.0	275.0	312.5	325.0	350.0	375.0	400.0	425.0	450.0	450.0
	45	287.5	300.0	325.0	350.0	362.5	400.0	425.0	450.0	450.0	450.0

Table 1c PIVET rFSH Dosing Chart suits Gonal-F, Puregon and Elonva											
AMH		5.0-9.9 pmol/L					<5.0 pmol/L				
AFC*		D (5-8 follicles)					E (≤4 follicles)				
BMI kg/m²		16-17	18-19	20-21	22-29	30-35	16-17	18-19	20-21	22-29	30-35
Age (years)	20	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0
	21	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0
	22	150.0	162.5	162.5	175.0	175.0	187.5	187.5	187.5	200.0	200.0
	23	162.5	162.5	175.0	175.0	187.5	187.5	187.5	200.0	200.0	212.5
	24	162.5	175.0	175.0	187.5	187.5	187.5	200.0	200.0	212.5	212.5
	25	162.5	175.0	175.0	187.5	187.5	200.0	200.0	200.0	212.5	212.5
	26	175.0	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0
	27	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0

	28	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5
	29	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	250.0
	30	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	250.0	262.5
	31	200.0	200.0	212.5	212.5	225.0	237.5	250.0	262.5	275.0	287.5
	32	212.5	225.0	237.5	250.0	262.5	275.0	287.5	300.0	312.5	325.0
	33	225.0	237.5	250.0	262.5	275.0	287.5	300.0	312.5	325.0	337.5
	34	250.0	262.5	275.0	287.5	300.0	325.0	350.0	375.0	400.0	425.0
	35	275.0	287.5	300.0	325.0	350.0	362.5	375.0	400.0	425.0	450.0
	36	275.0	287.5	300.0	325.0	350.0	375.0	400.0	425.0	450.0	450.0
	37	300.0	325.0	350.0	362.5	375.0	400.0	425.0	450.0	450.0	450.0
	38	325.0	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0
	39	350.0	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0
	40	375.0	400.0	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
	41	425.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
	42	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
	43	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
	44	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0
	45	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0	450.0

Increased basal FSH and Smokers Where FSH is less than 8 IU/L, with no history of smoking, use values as shown Smokers move 2 columns to the right Where FSH is between 8 and 12 IU/L, with no history of smoking, move one column to the right Smokers move two columns to the right Where FSH is greater than 12 IU/L, move two columns to the right Smokers and non-smokers read same column *Antral Follicle Count based on number of antral follicles <1.0 cm	Standard Dosing as above Aiming for 8-12 oocytes Oocyte Donors & Oocyte Banking Aiming for 10-15 oocytes, move four columns to the right Consider GnRH antagonist Trigger if >10 follicles e.g., Tryptorelin 100 μg X2
Colour Scheme 12.5 IU increments suits Gonal-F pen 25 IU increments also suits Puregon pen Elonva: 1x100 μg for weight ≤60 kg 1x150 μg for weight >60 kg	

Oocyte pick-up is conducted with a Cook single-lumen aspirating needle where follicle numbers exceed 5, and the PIVET-Cook double lumen needle where follicle numbers are ≤5 to enable combined aspiration and flushing [7,45]. From the laboratory perspective, PIVET has increasingly applied a blastocyst culture system (90% of embryos) followed by a SET regimen (91% of cases) and commitment to cryopreservation by vitrification using the Kuwayama method [50]. Under an ANZARD initiative, the results from all participating IVF units in Australia and New Zealand can now be

viewed [51] and reveals that PIVET's IVF program generates live birth productivity rates [52] in the highest quartile across all age groups of women. These high live birth productivity rates are achieved whilst recording multiple pregnancies in <5% of births (being twins only) and complications such as severe ovarian hyperstimulation syndrome (OHSS) requiring hospitalization occurring in only 1/ 1000 OPU cases.

2.2. Indications for ICSI

The range of indications for ICSI at PIVET includes male factor causes and extends across a range of non-male factor scenarios according to the recently reported study [43].

2.2.1. Male infertility factors

Male infertility is diagnosed according to the semen parameters defined by the WHO laboratory manual of 2010 [53] which delineates those samples with oligozoospermia, asthenozoospermia or teratozoospermia, and combinations of these, particularly that known as OAT (oligo-astheno-teratozoospermia) syndrome. However, PIVET also includes those men with identifiable anti-spermatozoal antibodies in their semen (IgG levels>20%) and those with significant levels of DNA fragmentation (≥15%) identified by the Halo test [54]. Male factor infertility also includes those cases requiring surgical sperm retrieval [20] including microsurgical epididymal sperm aspiration (MESA), percutaneous epidydimal sperm aspiration (PESA) and testicular sperm aspirations (TESA and micro-TESE) as well as Vasal Flush procedures, mostly applied for males with spinal injury [36]. Although PIVET does not apply direct testing of semen for ROS, cases of obstructive azoospermia are considered likely to have excessive ROS as an underlying reason for poor fertilizing capacity.

Clinical conditions of the male are also considered for ICSI. These include morbid obesity, the presence of clinically detectable varicocele, the presence of ejaculatory disorders, a history of maldescent of the testis, a history of epididymitis or testicular torsion, and any orchidopexy procedure. 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, along with males who use recreational drugs or are exposed to chemicals and heavy metals in "risky" occupations such as welding [56], are also encouraged to utilize ICSI.

2.2.2. Female factor infertility

Advanced female age as well as reduced ovarian reserve are inter-connected to lower antral follicle counts (AFCs) and low serum anti- Mullerian hormone (AMH). These combinations are closely associated with poor IVF prognosis [57], 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 those with diminished ovarian reserve [58].

It was reported by Diedrich's group that where <4 oocytes are re- covered, ICSI guarantees a successful treatment outcome more often than IVF and encourages the idea of milder forms of stimulation [59]. Our clinic has adopted a milder stimulation policy in recent years such that many women will now generate <5 oocytes where ICSI provides a greater chance of generating embryos for transfer, especially if few oocytes [60] or only a single oocyte is retrieved [61]. 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. The consequential effect is reduced or failed fertilization [62] and this appears to be related to the degree of response to gonadotrophin stimulation. Some zona problems can be encountered leading to reduced or absent sperm binding and these can mostly be resolved by ICSI [63].

2.2.3. Unexplained or poorly explained 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" [64]. Reduced fertilization rates <50% of mature oocytes in either an IVF- all or an IVF-ICSI Split "trial" indicates a need to apply ICSI for future IVF-related procedures [65]. The idea of applying an IVF-ICSI Split approach as a diagnostic exercise for all IVF-naïve cases of unexplained infertility or intrauterine insemination (IUI) failures has been demonstrated to be a cost-effective approach in the long term [66], one that is favored at PIVET.

2.2.4. The IVF-ICSI Split methodology

Women who are IVF-naïve are encouraged to undertake an IVF-ICSI Split technique on their very first IVF cycle if there is no identifiable male factor and the female infertility is either tubal or unexplained. Such cases could be expected to have normal fertilization potential, albeit that our extensive historical experience exceeding 45 years indicates that poor fertilization (fewer than 50% of oocytes) and complete failed fertilization will occur in ~15% (poor fertilization) and ~5% (failed fertilization) of cases respectively [40]. The IVF-ICSI Split technique is applied when ≥4 mature oocytes are retrieved at OPU and oocytes are distributed randomly for IVF or ICSI, prior to cumulus stripping for the ICSI cases. Where <4 mature oocytes are recovered, all oocytes are allocated to ICSI. Although this dataset covers the period from January 2011 to 2019 with outcomes tracked through 2020, the majority of the IVF-ICSI Split group were conducted in the years from 2014 (following unexpected cases of complete fertilization failure) when the practice has become routinely recommended for unexplained infertility.

2.2.4.1. Oocyte distributions

OCCs are graded immediately after OPU applying a longstanding protocol [67] assessing the cumulus cell numbers and tightness of their distribution along with an assessment of the density and tightness of the coronal cells surrounding the oocyte. Thereafter the OCCs are randomly distributed between IVF and ICSI with an equivalent distribution of OCC gradings between the groups. Two embryologists are involved in this process to ensure fairness of distribution between the groups.

2.2.4.2. Oocytes allocated to IVF

Oocytes for IVF were maintained as OCCs and 3-5 hours after OPU were placed in Fertilization Medium (Quinn's Advantage) along with 100,000 spermatozoa prepared 2-4 hours earlier. The sperm preparation involved centrifugation through a 2-layered colloidal silica suspension (Pure sperm 40/80; Nidacon, Sweden). The incubation conditions were within Minc solid-state incubators (COOK, Australia) at 37^0C in microdroplets under oil (Sage; Origio, Australia) which had been equilibrated overnight and perfused with humidified triple gas - 5% O_2, 6% CO_2 and 89% N_2.

2.2.4.3. Oocytes allocated for ICSI

Three to five hours after OPU, the OCCS 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 seconds to disperse the cumulus cells and thereafter subjected to fine needle pipetting to partially strip the remaining cumulus and coronal cells [68]. This was performed within a humidified workstation chamber gassed with 6% CO_2 in air within Quinn's Fertilization medium. Only oocytes at the metaphase II (MII) stage were accepted for ICSI. Those at MI or germinal vesicle (GV) stage, those showing a cracked zona pellucida or those oocytes displaying signs of degeneration are excluded from fertilization attempts.

2.2.4.4. Oocyte pronuclear-stage checks

For both IVF and ICSI fertilized oocytes, the pronuclear-stage checks were performed at 16h after overnight incubation in Minc solid-state chambers. Fertilization was reported as 2 PNs per oocytes collected, per oocyte allocated and per injected oocyte (i.e. per metaphase MII 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. All 2 PNs 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 performed.

2.2.4.5. Oocyte fertilization rates

For ICSI, the fertilization rate was recorded as those oocytes identified as 2 PNs per oocyte injected. For IVF, the actual fertilization rate was recorded as those oocytes identified as 2 PNS per OCCs inseminated. A second fertilization rate was also recorded being an adjusted rate as those oocytes identified as 2 PNS per OCCs inseminated, applying the same denominator as identified for ICSI. This enables an equivalent fairer comparison for statistical evaluation.

2.2.5. Intrauterine insemination failures

Infertile women who had failed to achieve a pregnancy following 2–6 cycles of intrauterine insemination (IUI) were advised to consider ICSI or at least IVF-ICSI Split from our internal studies [45] and indicated by others [66,68,69]. 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 fertilization failure were significantly higher (13.4% vs 2.9%; $p < 0.001$) causing a change in policy to recommend ICSI or IVF-ICSI Split in such cases.

2.2.6. Genetic analysis of embryos

Where preimplantation diagnosis (PGD) and screening (PGS) was applied, the current recommendations are to utilize ICSI to avoid DNA contamination of the embryo biopsy specimens (either blastomeres or trophoblast specimens) from non-fertilizing sperm adherent to the zona pellucida. This is recommended by the ESHRE PGD Consortium [70].

2.2.7. 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 a slow-freeze technique was applied [45].

2.3. Indications for IVF Only

Following our earlier study on IVF-ICSI Split, 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 the collection of >4 oocytes from the woman. Nonetheless, occasionally couples with definable male factor infertility have requested IVF Only or IVF-ICSI Split to determine if any IVF ('natural fertilization") embryos could be created. This is enabled at PIVET with first preference for transfer given to the IVF-generated embryos.

2.4. Freeze-all embryos policy

To avoid the risk of severe OHSS, the PIVET algorithm is applied for FSH-dosing meaning most women generate 10±2 follicles with a matching serum estradiol level (E2) of 10,000±2000 pm/L such that 8-12 oocytes are recovered at oocyte pick-up (OPU). Where >12 oocytes are recovered, women commence an increased monitoring protocol and where ≥15 oocytes are recovered, a freeze-all protocol is advised (and is mandatory for ≥20 oocytes) with the luteal phase managed by medroxyprogesterone (MPA) substitution and dopamine receptor antagonist (cabergoline) to minimize the symptoms of OHSS [45].

2.5. Embryo transfer policy

Throughout the decade of this study, the protocol has continually advanced towards single blastocyst transfers for both fresh (ET) and cryopreserved (FET) embryos. The criterion is mandatory for all women <40 years having up to 3 failed IVF±ICSI treatments and remains the preferred protocol for all cases across the treatment ages (to age 50 years). Currently blastocyst transfers are conducted in >90% of embryo transfers and the SET rate is >95%, hence the multiple pregnancy rate is <5%, mainly monozygotic twinning. The embryo transfers are conducted under trans-vesical abdominal ultrasound using the Cook K-JETS catheter system whereby a mid-high fundal "flash" is identified.

2.6. Pregnancy definition

At PIVET pregnancy is denoted by an elevated B-HCG level along with raised estradiol-17B (E2) and progesterone (P4) undertaken ~19 days after the OPU procedure; denoted as week-4 [45]. At PIVET all pregnancies are monitored each week until week-8 (6 weeks post-OPU). Clinical pregnancy is defined by a rising b-HCG from week-4 to week-5, with a definitive ultrasound at week-7 demonstrating an intra-uterine gestational with a viable fetus. Pregnancy losses are defined after week-5 and include pregnancies of unknown location (PUL) which may demonstrate spontaneous hormonal "fade-out" or be given methotrexate (MTX) to enhance the process when a clear intra-uterine gestation cannot be demonstrated. At week-7 pregnancies with a non-viable gestational sac in-utero are encouraged to have an evacuation procedure (along with cytogenetic assessment of the products of conception). Those without an intra-uterine gestational sac but persisting BHCG elevation at week are committed to laparoscopic evaluation to manage a probable ectopic gestation (or have intra-cornual MTX administered). PIVET has an active luteal-support approach requiring mid-luteal and early pregnancy P4 levels to record ≥60 nmol/L [45]. High-risk pregnancies and those with threatened miscarriage were offered medroxyprogesterone acetate during the first trimester, continuing to gestational age 35 weeks where pre-term delivery has occurred previously. We have recently published safety and beneficial outcomes from this strategy [71].

2.7. Live birth, pregnancy loss and fetal abnormality

At PIVET a dedicated midwife manages the pregnancy register and inputs key data into the Filemaker database. She establishes a close relationship with each of the women achieving pregnancy and receives information concerning early pregnancy losses, preterm deliveries, and any adverse event. Within one week after the expected date of delivery (EDD), contact is made with the obstetrician as well as the woman. Live birth (LB) deliveries are categorized as very early preterm, preterm, and full term if the number of gestation days was 140–195, 196–258 and ≥259 days, respectively. Pregnancy loss (PL) is categorized as either early (49 to <140 days; EPL, miscarriage) or later; sub-categorized as preterm stillbirth, or full-term stillbirth if the fetus was lost/delivered during gestation days 140–258 and ≥259 days, respectively. Each fetal abnormality is categorized according to the International Classification of Disease (ICD) code, and then sub-categorized into the related anatomical system including neural, eye defects, cardiovascular, gastrointestinal, urogenital, musculo-skeletal, skin, chromosomal and any other.

2.8. Statistical evaluation

Data from the Filemaker Pro (Apple Inc, USA) database were extracted into Microsoft Excel spreadsheets and assorted according to the relevant tests. The Flow Sheet was created using Microsoft Word for Mac v 16.54. Comparisons between Groups were analyzed in 2x2 contingency tables, mainly by Fisher's exact test, or by Chi-squared applying Yates' continuity correction factor for the larger data sets. Following corrections, probability values of $p<0.05$ were considered significant for any test. Apart from the Flow Sheet, the Figures displayed in this study are derived from Excel v 16.44 (Microsoft 2020), thereafter exported as Word files.

2.9. Abbreviations applied in this report

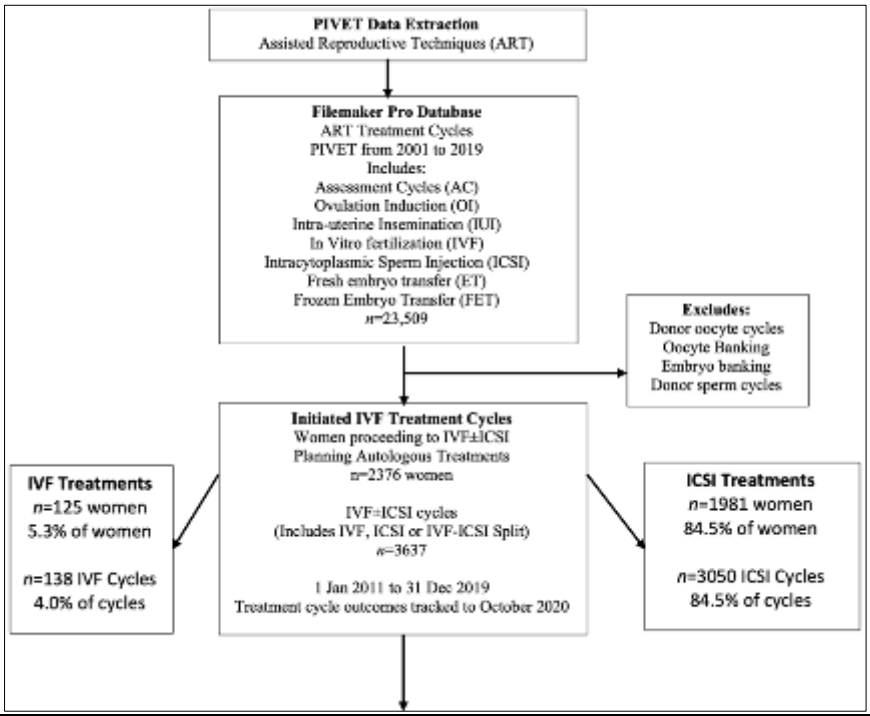
ART	Assisted reproduction treatments/ technologies
Fert Mode	Fertilization by IVF or by ICSI mode
Cancel, %	cancellation rate
O	oocyte/s
OPU	oocyte pick-up procedure
O Utn %	oocyte utilization rate
E Utn %	embryo utilization rate
E Fz %	embryo cryopreservation (frozen) rate
ET	Fresh embryo transfer
FET	Frozen embryo transfer
Trans.	Transfer/s
Preg/In %	pregnancy rate per initiated cycle
Preg/O %	pregnancy rate per oocyte pick-up (OPU)
Preg/ET %	pregnancy rate per embryo transfer procedure
EPL %	early pregnancy losses from week 5 (miscarriage rate)
LB/In %	live birth rate per initiated cycle
LB/O %	live birth rate per oocyte pick-up (OPU)
LB/ET %	live birth per embryo transfer procedure
LBPR	live birth productivity rate (live births per fresh and frozen ETs)
IVF	*In vitro* fertilization (insemination of OCC)
ICSI	Intracytoplasmic sperm injection
IVF-ICSI Split	OCCs randomised to IVF and ICSI modes
OCC	Oocyte cumulus complex
MII Oocyte	Oocyte at metaphase II stage; signifies mature
2 PN	Oocyte displaying 2 pronuclei; signifies fertilized
AFC	antral follicle count (small follicles <10mm)
AMH	anti-Mullerian hormone
BMI	Body mass index kg/m^2

FSH	Follicle stimulating hormone (serum measurement)
PIVET	registered acronym from programmed IVF & ET
Stats	statistics
n.s.	not significant
yrs	years
#	total

3. Results

Across the period 2011 to 2019, 2376 infertile women initiated 3767 IVF±ICSI treatment cycles at PIVET where autologous oocytes were utilized to generate embryos intended for transfer with the view of generating healthy children for oneself. (Whilst this includes women accessing donor sperm, the 242 treatment cycles from 233 women utilizing the IVF-ICSI Split protocol is reserved for those women with a male partner who has a normal semen analysis and the couple being categorized as having unexplained infertility). This included the idea of cryopreservation of embryos and maximizing the productivity rate from a single initiated IVF cycle as reported in this study. Therefore, cycles involving donor sperm, donor oocytes and donor embryos have been excluded. Also cycles involving oocyte or embryo banking for future potential use have also been excluded.

Figure 1 Flowsheet depicting the derivation of 3637 IVF±ICSI treatment cycles from 2376 women across the period 1 January 2011 until 31 December 2019 with pregnancy outcomes tracked through 2020. The treatments are sub-categorized according to the insemination process; IVF, ICSI or IVF-ICSI Split modes. The IVF-ICSI Split cycles are further categorized according to those reaching a fresh ET procedure and those having FET procedures following a freeze-all cycle.



All Autologous IVF-ICSI Split Cycles during 2011-2019

Cycles classified retrospectively as IVF or ICSI according to derivation of embryo transferred

Ages	**<35 years**			**35-39 years**			**40-44 years**			**≥45 years**			**Totals**		
Mode	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF
Cycles	144	65	58	65	36	29	18	11	1	1	1	1	242	113	92
Women	141	64	57	75	35	28	16	10	1	1	1	1	233	110	90

All Autologous IVF-ICSI Split Cycles during 2011-2019 with Fresh ET ± subsequent FETs *"Best" embryo selected for transfer according to morphological criteria*															
Ages	**<35 years**			**35-39 years**			**40-44 years**			**≥45 years**			**Totals**		
Mode	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF
Cycles	121	65	58	65	36	29	14	11	1	1	1	1	199	113	92
Women	119	64	57	60	35	28	13	10	1	1	1	1	193	110	90

All Autologous IVF-ICSI Split Cycles during 2011-2019 with Freeze-All, ± subsequent FETs															
Ages	<35 years			35-39 years			40-44 years			≥45 years			Totals		
Mode	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF
Cycles	23	23	19	16	15	13	3	1	0	0	0	0	42	39	35
Women	23	23	19	15	14	13	3	1	0	0	0	0	41	38	35

From the Flow sheet (Figure1) most treatments were undertaken utilizing the ICSI technique for fertilization - 1981 of the women (84.5%) initiated 3050 cycles (88.9%) for insemination by ICSI. Hence, only a smaller proportion of women conducted their treatments by IVF insemination only – 125 of the women (5.3%) initiated 138 cycles (4.0%) for insemination by traditional IVF. These women had mostly been shown to have satisfactory fertilization (≥50% of oocytes) on previous treatment cycles. Therefore, IVF-naïve women with tubal or unexplained infertility were encouraged to undertake IVF-ICSI Split treatments hence 233 women (9.8%) initiated 242 (6.7%) treatment cycles where ≥4 oocytes were randomly allocated to either IVF or ICSI insemination. Cases are then categorized according to the derivation of the embryo transferred (being IVF-generated or ICSI-generated). The number of women and their IVF±ICSI cycles are shown in specific display boxes (covering the 242 total treatment cycles, the 199 treatment cycles having a fresh embryo transfer, and the 42 treatment cycles having freeze-all embryos, some of which proceeded to subsequent FETs).

Table 2 Summarizes the raw data outcomes of 242 IVF-ICSI Split Cycles from 233 women who reached OPU

Autologous IVF-ICSI Split Cycles during 2011-2019 – numbers					
Age groups	**<35 yrs**	**35-39 yrs**	**40-44 yrs**	**≥45 yrs**	**Total**
Treatment Cycles	144	79	18	1	242
Women treated	141	75	16	1	233
Cancelled cycles	0	0	0	0	0
#OPU	144	79	18	1	242
Oocytes Collected (OCCs)	2046	1073	223	4	3346
Oocytes Inseminated IVF	965	496	107	2	1570
Oocytes Injected ICSI	873	446	88	2	1409
#2PN	1249	631	124	2	2019
- 2PN (ICSI Only)	729	360	64	1	1154
- 2PN (IVF Only)	521	283	60	1	865
#Freeze-all embryos	23	16	3	0	42
#ET cycles (Fresh ETs)	121	64	14	1	200

Total Fresh embryos transf.	124	69	17	2	212
- Fresh ICSI transfer	65	40	12	1	118
- Fresh IVF transfer.	59	29	5	1	94
# Embryos Frozen Total	519	251	27	0	797
- ICSI Embryos Frozen	312	147	11	0	470
- IVF Embryos Frozen	207	104	16	0	327
FET Cycles	167	88	13	0	268
Total embryos at FETs	168	90	15	0	273
- ICSI embryo FETs	108	54	6	0	168
- IVF embryo FETs	168	100	16	0	284
Total Transfers Fresh/Frozen	288	151	27	2	468
Total Embryos F/Fz transfers	291	158	32	2	483
#Pregs Fresh only	61	19	3	0	83
- Pregs Fresh (ICSI)	30	11	1	0	42
- Pregs Fresh (IVF)	31	8	2	0	41
#Pregs Frozen only	84	41	2	0	127
- Pregs Frozen (ICSI)	66	36	1	0	103
- Pregs Frozen (IVF)	18	5	1	0	24
#Pregs Fresh/frozen	145	60	5	0	210
#Miscarriages	31	14	2	0	47
#Live Births from fresh ETs	54	15	1	0	70
#Babies from Fresh ETs	55	16	1	0	72
- Live Births (ICSI)	28	8	0	0	36
- Live Births (IVF)	26	7	1	0	34
#Live Births from FETs	61	31	2	0	94
#Babies from FETs	66	31	2	0	97
- Livebirths (ICSI) Fz	36	21	1	0	58
- Livebirths (IVF) Fz	30	19	1	0	50
#LiveBirths Fresh/Fz 1st Trans.	115	46	3	0	164
Total babies Fresh/Fz 1st Trans.	121	46	3	0	170

The laboratory data including oocyte numbers retrieved at OPU and the insemination outcomes (IVF vs ICSI) are depicted in Table 2. It can be seen that 3346 OCCs were recovered (13.8 oocytes/ OPU) and 2979 were deemed suitable for a fertilization procedure, allocating 1570 to IVF and 1409 to ICSI. It is shown that the 2PN (pronuclear stage) which identified normal fertilization was identified in 2019 of the 2979 oocytes inseminated (by IVF or ICSI) being 67.8% overall. This means 11.0% oocytes are not inseminated as they have not reached the MII stage of maturity or have fractured zonae, possibly a reflection of using the double-lumen needle for follicle flushing which enables a very high oocyte recovery rate approaching 100% for follicles ≥14mm [7]. However, the fertilization rate varied from 865 2PNs arising from 1570 oocytes undergoing IVF insemination (55.1%) to 1154 of 1409 oocytes (81.9%) having ICSI insemination. For comparable matching of oocytes, the IVF rates are adjusted to 865 of 1409 oocytes (61.4%) to match those MII oocytes defined for ICSI. Irrespective of the adjustment, these fertilization rates were highly significant across

the entire age range groupings, in favor of ICSI (p<0.0001). However, when analyzing the fertilization rates for individual age groupings, this highly significant difference was shown only for those women aged <40 years (Table 3).

Table 3 Shows the rates and statistical significance of the outcomes of 242 IVF-ICSI Split Cycles from 233 women who reached OPU

All Autologous IVF-ICSI Split Cycles during 2011-2019 – rates %															
Ages	<35 years			35-39 years			40-44 years			≥45 years			Totals		
Fert mode	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF
Cycles	144	65	58	65	36	29	18	11	1	1	1	1	242	113	92
Women	141	64	57	75	35	28	16	10	1	1	1	1	233	110	90
Cancel %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O/OPU	14.2	12.1	12.9	13.6	11.6	12.2	12.4	11.5	15.5	4.0	4.0	4.0	13.8	11.8	12.7
Fert %*	61.0	84.1	64.6	58.8	83.8	66.7	55.6	76.4	60.0	50.0	50.0	50.0	60.0	83.2	65.4
Stats:	*p<0.0001*			*p<0.0001*			*n.s.*			*n.s.*			*p<0.0001*		
O Utn %	31.3	31.3	34.9	29.6	26.7	30.8	19.7	20.5	22.6	50.0	50.0	50.0	30.0	46.8	42.7
E Utn %	51.3	51.9	53.4	50.4	46.9	49.8	35.5	34.7	42.4	100	100	100	50.1	48.9	51.9
E Fz %	41.5	37.9	41.1	39.6	29.7	36.1	21.8	17.3	24.2	0.0	0.0	0.0	39.6	33.4	38.8
PP/ ET %	49.8	42.6	56.8	38.0	44.9	33.8	15.6	10.0	25.0	0.0	0.0	0.0	43.5	40.1	46.8
PP/Init %	100.7	89.2	108.6	75.9	86.1	75.9	27.8	18.2	50.0	0.0	0.0	0.0	86.8	80.5	94.6
PP/OPU %	100.7	89.2	108.6	86.1	86.1	75.9	27.8	18.2	50.0	0.0	0.0	0.0	86.8	80.5	94.6
EPL %	21.4	12.1	25.4	23.3	27.3	27.3	40.0	50.0	50.0	0.0	0.0	0.0	22.4	18.7	26.4
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
LBR/ET%	39.2	37.5	42.3	29.1	31.9	24.6	9.4	5.0	12.5	0.0	0.0	0.0	33.7	32.6	34.4
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
LBP/ In %	79.2	78.5	81.0	58.2	55.2	55.2	16.7	9.1	25.0	0.0	0.0	0.0	67.4	65.5	69.6
LBP/OPU	79.2	78.5	81.0	58.2	55.2	55.2	16.7	9.1	25.0	0.0	0.0	0.0	67.4	65.5	69.6
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		

*Fertilization rate for IVF: denominator adjusted from total 425 oocytes, to 358 oocytes reflecting likely number of MII oocytes (presumed to be the same as ICSI group).

The utilization rates of those embryos arising from the fertilized oocytes are shown in Table 3 which denotes them as either IVF or ICSI-derived. A proportion of the embryos were transferred in fresh cycles (total n=200 embryos in 200 ET cycles) whilst a larger proportion were cryopreserved (total n=797 embryos; 470 derived from ICSI and 327 derived from IVF). From the combined total of 468 ET and FET transfers a total 483 embryos were transferred indicating a 96.9% SET rate. The 3.1 % of women having 2 embryos transferred were all aged over 40 years with limited opportunity for repeat ART procedures. The embryo utilization rates across the age groups are shown in Table 3 where the rates were ~45% with no significant differences across the groups <45 years. However, in the oldest group of women ≥45 years there was an apparently higher utilization for IVF-derived embryos, but this is likely an aberration related to the low numbers in this group as well as the determined attempt to give such women "every chance" despite low quality of their embryos. With respect to pregnancies arising from the respective ICSI-generated or IVF-generated embryos, there were no significant differences. This finding carried through to pregnancy outcomes with similar rates of miscarriage and live births across the age groupings as well as the total population of 233 women having 242 treatment cycles.

Table 4 Summarizes the raw data outcomes of 200 IVF-ICSI Split Cycles from 193 women who reached the stage of a fresh ET. The data excludes 42 women who had cycles where all embryos were cryopreserved (freeze-all)

Autologous IVF-ICSI Split Cycles excluding 42 Freeze-alls – numbers					
Age groups	**<35 yrs**	**35-39 yrs**	**40-44 yrs**	**≥45 yrs**	**Total**
Treatment Cycles	121	63	14	2	200
Women treated	119	60	13	1	193
Cancelled cycles	0	0	0	1	1
#OPU	121	63	14	1	199
Oocytes Collected (OCCs)	1503	752	182	4	2441
Oocytes Inseminated IVF	710	346	86	2	1144
Oocytes Injected ICSI	657	318	73	2	1050
#2PN	940	444	105	2	1504
- #2PN (ICSI Only)	546	258	53	1	858
- #2PN (IVF Only)	395	198	52	1	646
#Freeze-all embryos	0	0	0	0	0
#ET cycles (Fresh ETs)	121	63	14	1	199
Total Fresh embryos transf.	125	68	17	2	212
- Fresh ICSI transfer.	65	40	12	1	118
- Fresh IVF transfer.	59	29	5	1	94
# Embryos Frozen Total	370	150	21	0	541
ICSI Embryos Frozen	226	89	8	0	323
IVF Embryos Frozen	144	61	13	0	218
FET Cycles	118	62	8	0	188
Total Frozen embryos trans.	119	62	9	0	190
- Frozen ICSI embryos trans.	84	39	3	0	126
- Frozen IVF embryos trans.	108	57	13	0	178
Total Transfers Fresh/frozen	242	130	26	2	400
Total Embryos F/fr transfer.	492	217	38	2	749
#Pregs Fresh only	61	19	3	0	83
- Pregs Fresh (ICSI)	30	11	1	0	42
- Pregs Fresh (IVF)	31	8	2	0	41
#Pregs Frozen only	57	33	1	0	91
- Pregs Frozen (ICSI)	46	29	0	0	75
- Pregs Frozen (IVF)	11	4	1	0	16
#Pregs Fresh/frozen	118	52	4	0	174
#Miscarriages	23	13	2	0	38
#Live Births from fresh ETs	54	15	1	0	70
#Babies from fresh ETs	55	15	1	0	71

- Live Births (ICSI) Fresh	28	8	0	0	36
- Live Births (IVF) Fresh	26	7	1	0	34
#Live Births from FETs	42	24	1	0	67
Total Babies from FETs	43	25	1	0	69
- Live Births (ICSI) Fz	19	15	0	0	34
- Live Births (IVF) Fz	16	16	1	0	33
#LiveBirths from Fresh/Fz	96	39	2	0	137
- Live Births (ICSI) Fr/Fz	47	23	0	0	70
- Live Births (IVF) Fr/Fz	42	23	2	0	67
Total babies from Fresh/Fz	91	47	2	0	140

Analyzing the laboratory and clinical outcomes from those women who had fresh embryo transfers separately (i.e., excluding those who had a high response with oocyte numbers >15, resulting in a "freeze-all" cycle), in Table 4 it can be seen that 193 women reached the stage of OPU but one cycle in a woman aged 45-years, was cancelled at the stage of Trigger because of an uncertain response with probable premature ovulation. The 199 cycles yielded 2441 OCCs of which 1050 were allocated to ICSI and 1144 were inseminated by IVF (10.1% discarded. Applying the same denominator of 1050, the fertilization rates were 858; 81.7% for ICSI and 646; 61.5 % for IVF (p<0.0001).

Table 5 Shows the rates and statistical significance of the outcomes of 200 IVF-ICSI Split Cycles from 193 women who reached the stage of a fresh ET. The data excludes 42 women who had cycles where all embryos were cryopreserved (freeze-all)

Autologous IVF-ICSI Split Cycles excluding 42 Freeze-all – Rates %															
Ages	<35 years			35-39 years			40-44 years			≥45 years			Totals		
Adj	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF
Cycles	121	65	58	65	36	29	14	11	1	2	1	1	200	113	92
Women	119	64	57	60	35	28	13	10	1	2	1	1	193	110	90
Canc %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.0
O/OPU	12.4	12.1	12.9	11.9	11.6	12.2	13.0	11.5	15.5	4.0	4.0	4.0	12.3	11.8	12.7
FertRate %	62.5	84.1	64.6	59.0	83.8	66.7	57.7	76.4	69.0	50.0	50.0	50.0	61.1	83.2	65.4
Stats:	*p<0.0001*			*p<0.0001*			*n.s.*			*n.s.*			*p<0.0001*		
O Utn %	32.7	31.3	34.9	28.9	26.7	30.8	20.9	20.5	22.6	50.0	50.0	50.0	30.7	28.9	33.1
E Utn %	52.3	51.9	53.4	48.9	46.9	49.8	36.2	34.7	42.4	100	100	100	50.2	48.9	51.9
E Fz %	39.3	37.9	41.1	33.6	29.7	36.1	20.0	17.3	24.2	0.0	0.0	0.0	36.2	33.4	38.8
PP/ET %	48.8	42.6	56.8	40.0	44.9	33.8	15.4	10.0	25.0	0.0	0.0	0.0	43.5	40.1	46.8
PP/Init %	97.5	89.2	108.6	82.5	86.1	75.9	28.6	18.2	50.0	0.0	0.0	0.0	87.4	80.5	94.6
PP/ OPU %	97.5	89.2	108.6	82.5	86.1	75.9	28.6	18.2	50.0	0.0	0.0	0.0	87.4	80.5	94.6
EPL %	19.5	12.1	25.4	25.0	27.3	27.3	50.0	50.0	50.0	0.0	0.0	0.0	21.8	17.6	26.4
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
LBP/ET %	39.3	37.5	42.3	30.0	33.3	24.6	7.7	5.0	12.5	0.0	0.0	0.0	34.0	33.0	34.4
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		

LBP/Init %	79.2	78.5	81.0	58.2	55.2	55.2	16.7	9.1	25.0	0.0	0.0	0.0	67.4	65.5	69.6
LBP/OPU %	78.5	78.5	81.0	61.9	63.9	55.2	14.3	9.1	25.0	0.0	0.0	0.0	68.3	66.4	69.6
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		

Despite the significantly higher fertilization numbers and rates shown in Table 4, the embryo utilization rates (embryos resulting in ETs or cryopreserved) were similar between the IVF-generated and the ICSI-generated. Hence, as shown in Table 5, the pregnancy productivity rates (from fresh ETs and FETs), the miscarriage rates (EPLs) and the live birth productivity rates were not significantly different, neither for the total group nor the individual age groupings.

Table 6 Summarizes the raw data outcomes of 42 IVF-ICSI Split Cycles from 41 women who reached OPU but where all resulting embryos were cryopreserved (freeze-all) because of a high risk of OHSS. Such women subsequently had FET procedures

Autologous IVF-ICSI Split Cycles: 42 Freeze-all – numbers					
Age groups	<35 yrs	35-39 yrs	40-44 yrs	≥45 yrs	Total
Treatment Cycles	23	16	3	0	42
Women treated	23	15	3	0	41
Cancelled cycles	0	0	0	0	0
#OPU	23	16	3	0	42
Oocytes Collected (OCCs)	543	321	39	0	903
Oocytes/ OPU	23.6	20.1	13.0	0	21.5
Oocytes Injected by ICSI	216	128	14	0	358
Oocytes Inseminated by IVF	255	150	20	0	425
#2PN	309	187	19	0	515
- #2PN (ICSI)	183	102	11	0	296
- #2PN (IVF Only) *	126	85	8	0	219
Fertilization rate %	65.6	67.2	55.9	0	65.7
- Fert by ICSI (296/358)	84.7	79.7	78.6	0	82.7
- Fert by IVF (219/358)*	58.3	66.4	57.1	0	61.2
# Embryos Frozen	149	101	6	0	256
- ICSI Embryos Fz	86	58	3	0	147
- IVF Embryos Fz	63	43	3	0	109
FET Cycles	49	26	5	0	80
Total Frozen Embryos Transfers	49	28	6	0	83
- FET ICSI embryos	24	15	3	0	42
- FET IVF embryos	60	43	3	0	106
#Pregs Frozen only	27	8	1	0	36
- Pregs Frozen (ICSI)	20	7	1	0	28
- Pregs Frozen (IVF)	7	1	0	0	8
#Miscarriages	8	1	0	0	9

#Live Births from FETs	19	7	1	0	27
- Livebirths (ICSI) Frozen	9	4	1	0	14
- Livebirths (IVF) Frozen	10	3	0	0	13
Total Babies from FETs	21	7	1	0	29

*Fertilization rate for IVF: denominator adjusted from total 425 oocytes, to 358 oocytes reflecting likely number of MII oocytes (presumed to be the same as ICSI group).

Separately analyzing the data from those women who had a "freeze-all" treatment cycle, Table 6 indicates that 41 women had 42 cycles where 903 OCCs were recovered at OPU (21.5 oocytes/ OPU). These cycles were deemed high risk for OHSS. Of the 903 OCCs, 783 oocytes were utilized with 358 allocated to ICSI and 425 allocated to insemination by IVF (13.3% oocytes discarded. Applying the same ICSI denominator of 358, the fertilization rates were 82.7% for ICSI and 61.2% for IVF (p<0.0001).

Table 7 Shows the rates and statistical significance of the outcomes of 42 IVF-ICSI Split Cycles from 41 women who reached OPU but where all resulting embryos were cryopreserved (freeze-all) because of a high risk of OHSS. Such women subsequently had FET procedures

Autologous IVF-ICSI Split Cycles: 42 Freeze-all – Rates															
Ages	<35 years			35-39 years			40-44 years			≥45 years			Totals		
Fert Mode	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF	All	ICSI	IVF
Cycles	23	23	19	16	15	13	3	1	0	0	0	0	42	39	35
Women	23	23	19	15	14	13	3	1	0	0	0	0	41	38	35
Cancel %	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O/OPU %	23.6	23.6	24.4	20.1	21.1	21.9	13.0	23.0	13.0	0	0	0	21.5	22.6	22.5
Fert Rate %	65.6	84.7	58.3	67.2	79.7	66.4	55.9	78.6	57.1	0	0	0	65.7	82.7	61.2
Stats:	*p<0.0001*			*p<0.05*			*n.s.*			*n.s.*			*p<0.0001*		
O Utn %	27.4	27.4	29.8	31.5	31.6	32.3	15.4	17.4	15.4	0	0	0	28.3	28.7	30.0
E Utn %	48.2	48.2	49.6	54.0	53.8	54.8	31.6	33.3	31.6	0	0	0	49.7	49.9	50.8
E Fz %	48.2	48.2	49.6	54.0	53.8	54.8	31.6	33.3	31.6	0	0	0	49.7	49.9	50.8
Preg/In %	117.4	117.4	126.3	50.0	53.3	53.8	33.3	100	33.3	0	0	0	85.7	92.3	91.4
Preg/OPU %	117.4	117.4	126.3	53.3	53.3	53.8	33.3	100	33.3	0	0	0	85.7	92.3	91.4
Preg/ET %	55.1	55.1	55.8	28.6	28.6	29.2	16.7	33.3	16.7	0	0	0	43.4	45.0	43.8
EPL %	29.6	29.6	33.3	12.5	14.3	14.3	0.0	0.0	0.0	0	0	0	25.0	25.0	28.1
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
LB/ET %	38.8	38.8	37.2	25.0	25.0	25.0	16.7	33.3	16.7	0	0	0	32.5	33.8	31.5
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		
LB/Init %	82.6	82.6	84.2	43.8	46.2	46.2	33.3	100	33.3	0	0	0	64.3	69.2	65.7
LB/OPU %	82.6	82.6	84.2	43.8	46.7	46.2	33.3	100	33.3	0	0	0	64.3	69.2	65.7
Stats:	*n.s.*			*n.s.*			*n.s.*			*n.s.*			*n.s.*		

*Fertilization rate for IVF: denominator adjusted from total 425 oocytes, to 358 oocytes reflecting likely number of MII oocytes (presumed to be the same as ICSI group).

Again, despite the significantly higher fertilization numbers and rates shown in Table 6, the embryo utilization rates (embryos resulting in ETs or cryopreserved) were similar between the IVF-generated and the ICSI-generated. Hence,

as shown in Table 7, the pregnancy productivity rates (from fresh ETs and FETs), the miscarriage rates (EPLs) and the live birth productivity rates were not significantly different, neither for the total group nor the individual age groupings.

Table 8 Summarizes the raw data outcomes of 26 IVF-ICSI Split Cycles from 26 women who reached OPU and displayed fertilization in one arm only (IVF or ICSI) following random allocation of their oocytes

IVF-ICSI Split Cycles 2011-2019 Fertilization in one arm only - numbers		
	ICSI fertilization only	**IVF fertilization only**
Total initiated cycles	24	2
Women represented	24	2
Age range	23-42 years	35-38 years
Cancelled cycles	0	0
OPU procedures	24	2
Oocytes collected (OCCs)	284	14
Oocytes/ OPU (range)	5-27	5-9
Oocytes injected (ICSI)	128	6
Oocytes inseminated (IVF)*	142/ 128	7/5
Total 2PNs (ICSI-derived)	98	0
Total 2PNs (IVF-derived)	0	2
Fresh ET cycles	20	1
Embryos transferred at ET	22	1
Total embryos frozen	37	1
FET cycles	18	1 pending
Embryos transferred at FET	40	1 for donation
Total embryos utilized	59	2
Fresh ET pregnancies	5	0
FET pregnancies	9	Pending#
Total Fresh & Fz pregs	14	0 + 1 pending#
EPLs	2	0
Fresh ET live births	5	Nil?
FET Live births	7	Nil?
Total fresh & Fz live births	12	Nil?

*Denotes 142 OCCs/ assumes 128 at MII stage (as determined following cumulus stripping for ICSI; similar for 7/5. #One woman had freeze-all (deferring ET for pending myomectomy); high quality blastocyst (5BA by Gardner grading) frozen, now offered for donation.

Table 8 displays the laboratory and clinical outcomes from 26 women in both young and older age ranges who demonstrated fertilization in one arm only of their randomized oocyte allocation. Fertilization in only the ICSI arm occurred in 24 occasion (from 24 women) where the OPU range was 5-27 OCCs. Those women generated 98 embryos from 128 oocytes injected by ICSI (76.6% fertilization rate) but nil from 142 OCCs exposed to IVF (0/128 adjusted fertilization rate). Fertilization in only the IVF arm occurred on 2 occasions (from 2 women) where the OPU range was 5-9 OCCs. Those women generated only 2 embryos from 7 oocytes inseminated by IVF (28.6%, adjusted to 40.0% fertilization) but nil from 6 oocytes MII oocytes treated by ICSI (0.0% fertilization).

Table 9 The rates and clinical outcomes of 26 IVF-ICSI Split Cycles from 26 women who reached OPU and displayed fertilization in one arm only (IVF or ICSI) following random allocation of their oocytes

IVF-ICSI Split Cycles 2011-2019 Fertilization in one arm only - rates (%)		
	ICSI fertilization only	**IVF fertilization only**
Total initiated cycles	24	2
Women represented	24	2
Age range	23-42 years	35-38 years
Cancellation rate %	0.0	0.0
OPU procedures	24	2
Oocytes collected	284	14
Oocytes/ OPU mean (range)	11.8 (5-27)	7.0 (5-9)
Oocytes injected (ICSI)	128	5
Oocytes inseminated (IVF)*	142/ 128	7/5
Fertilization rate (ICSI) %	76.6	0.0
Fertilization rate (IVF) %	0.0	40.0
Oocyte utilization %	20.8	14.3
Embryo utilization %	60.2	100
Pregnancy rate Fresh ET	20.8	0.0
Pregnancy rate FET	50.0	Not utilized#
Live birth rate Fresh ET	20.8	0.0
Live birth rate FET	38.9	5BA embryo not utilized#
EPLs	14.3	No pregnancies
PPR /Initiated cycle	58.3	Nil?
PPR/ OPU	58.3	Nil?
PPR/ET	70.0	Nil?
LBP/Initiated cycle	50.0	Nil?
LBP/OPU	50.0	Nil?
LBP/ET	60.0	Nil?

*Denotes 142 OCCs/ assumes 128 at MII stage (as determined following cumulus stripping for ICSI; similar for 7/5. #One woman had freeze-all (deferring ET for pending myomectomy); high quality blastocyst (5BA by Gardner grading) frozen, now offered for donation.

The embryo utilization and clinical outcomes are shown in Table 9 where it can be seen that both oocyte and embryo utilization rates were comparable for both IVF and ICSI, but pregnancies only occurred in the ICSI group where live birth productivity outcome was 50.0% per initiated cycle. However, one of the 2 women in the IVF arm had her sole good-quality blastocyst embryo cryopreserved, pending a myomectomy procedure. However, this has not eventuated during the study period and the woman has offered the embryo for anonymous donation. If that embryo was given thew opportunity for FET, the possibility is that the potential live birth ratio could have been 12/24 (50%) for ICSI and 1 from 2 (50%) for IVF (no statistical difference).

Table 10 Summarizes the raw data and rates for the clinical outcomes from a further 86 FET cycles following the first ET or FET treatment cycle from the original 242 IVF-ICSI Split cycles

FET pregnancies following those from the First IVF-ICSI Split Cycle - numbers			
	ICSI-generated	**IVF-generated**	**Total**
Cycles with FET	38	44	86
Pregnancies n, % per ET	24 63.2%	8 18.2%	32
	p<0.0001		
EPLs n, %	3 12.5%	2 25.0%	5
Live births n, % per ET	21 55.3%	6 13.6%	27
Babies	22	6	28
	p<0.0001		

The 86 FET cycles shown in Table 10 denote secondary FET procedures following the first round ET plus FET from the 242 treatment cycles shown in Tables 2. The women had failure to achieve pregnancy in the first round or did achieve a pregnancy with either miscarriage or live birth outcomes. The main observation is that the pool of cryopreserved embryos from which this data is drawn is a secondary group, where the best quality embryos have already been sourced for the ET and FET treatments undertaken in the first round. It can be seen that 24 pregnancies arose from 38 FET cycles using ICSI-generated embryos and only 8 pregnancies arose from 44 FET cycles using IVF-generated embryos. The live birth rates were also significantly different being 55.3% for ICSI-generated embryos and 13.6% for IVF-generated embryos (p<0.0001).

Table 11 Summarizes the raw data and rates for the clinical outcomes from those embryos remaining in cryopreservation from the original 242 IVF-ICSI Split cycles. The potential outcomes are estimated from the actual rates arising in Table 3

242 Autologous IVF-ICSI Split Cycles – potential from remaining Fz embryos		
	ICSI-generated	**IVF-generated**
Total embryos in cryostorage	302 blastocysts	43 blastocysts
Age <35 years	204	39
- Pregs/ET 42.6%, 56.8%*	88 pregnancies	34 pregnancies
- LBR/ET 37.5%, 42.3%*	77 babies	17 babies
Age ≥35 years	98	4
- Pregs/ET 44.9%, 33.8%*	44 pregnancies	2 pregnancies
- LBR/ET 31.9%, 24.6%*	32 babies	1 baby
All women; potential pregnancies	132 pregnancies	36 pregnancies
All women; potential births	109 births	18 births
All women; potential babies	110 babies#	18 babies

*Rates derived from Table 3. #Includes 1 monozygotic twinning

By completion of the study period, January 2011 to December 2019, there were 345 blastocyst embryos remaining in cryostorage and which had been generated from the 233 women who had 242 IVF-ICSI Split treatment cycles. Table 11 shows the distribution of those embryos according to the women's age grouping and the derivation of the embryos, being ICSI-generated (n=302) or IVF-generated (n=43). The marked difference in embryo numbers between ICSI and IVF related to the highly significant fertilization rates, shown in Table 2 and Table 3. By utilization the pregnancy and live birth rates shown in Table 3, the potential clinical outcomes for those remaining embryos in cryostorage, has been

applied to project the likely potential livebirths in Table 11. There is likely to be 109 live births from ICSI and only 18 births from IVF, a significant difference for the 233 women having ART procedures ($p<0.0001$).

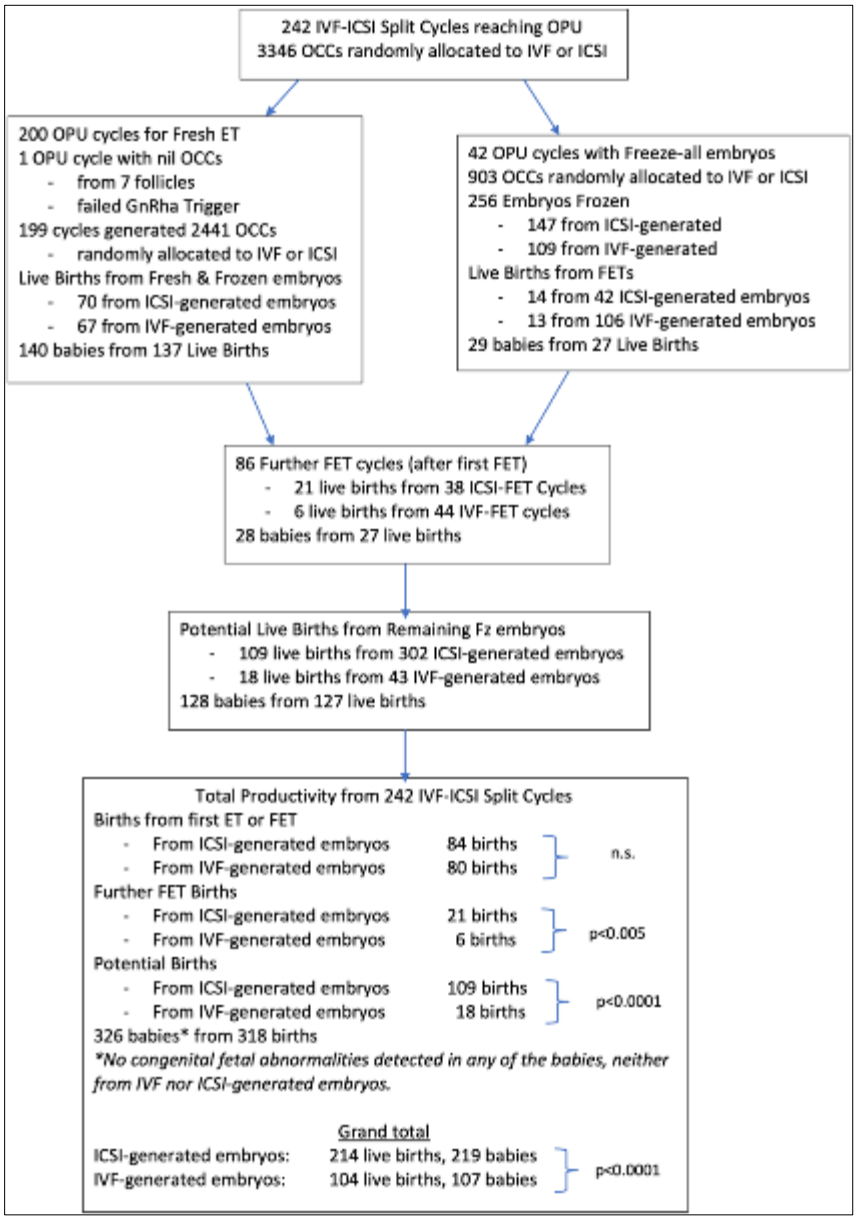
Table 12 Summarizes the raw data for the actual and potential clinical outcomes arising from the original 242 IVF-ICSI Split cycles and includes the total productivity from fresh ETs, the first FET treatment cycle, any subsequent FETS in the timeframe (2011-2019) as well as the potential productivity (pregnancies, live births and babies) from those embryos remaining in cryostorage. The statistical analysis is shown in Figure 2

242 Autologous IVF-ICSI Split Cycles – total productivity			
199 Cycles with fresh ET	**ICSI-generated**	**IVF-generated**	**Totals**
- Live births from ET*	36 births*	34 births*	70 births*
- Babies from ET	37 babies	34 babies	71 babies
- Live births from FET	34 births	33 births	67 births
- Babies from FET	35 babies	34 babies	69 babies
- Live births from ET + FET	70 births	67 births	137 births
- Babies from ET + FET	72 babies	68 babies	140 babies
42 Freeze-all Cycles			
- Live births, all FET	14 births	13 births	27 births
- Babies from FET	15 babies	14 babies	29 babies
242 Cycles – First pregnancy			
- Live births from ET & FET	84 births	80 births	164 births
- Babies	87 babies	83 babies	170 babies
242 Cycles + Subsequent pregs			
- Live births from FET	84 + 21 = births	80 + 6 births	191 births
- Babies	87 + 22 babies	83 + 6 babies	198 babies
Potential Cycles from Cryostorage	132 pregnancies	36 pregnancies	
+ Potential births from FET	109 births	18 births	127 births
Potential babies from FET	110 babies	18 babies	128 births
All live births (actual + potential)	84 + 21 + 109	80 + 6 + 18	318 births
Total babies (actual + potential)	109 + 110 babies	89 + 18 babies	326 babies

*6 women had 2 embryos transferred where each embryo was derived by ICSI or IVF.
The 2 singleton live births arising have been allocated according to the highest quality grading of the embryos, one by ICSI and one by IVF.
No congenital abnormalities were detected in any of the babies (neither IVF nor ICSI generated).

Compiling the live birth data from the various IVF-ICSI treatment groups shown in Table 2, Table 4, Table 6 and Table 8 enables a calculation of actual live births and babies born from this study. By adding in the potential data show in Table 10 enables a complete picture showing that 318 births generated 326 babies (Table 12) from 233 women who undertook 242 treatment cycles. This is shown in graphical form in Figure 1.

Figure 2 Flowsheet documenting the clinical outcomes indicating the derivation of live births and babies delivered arising from the primary data set of the 242 IVF-ICSI Split treatment cycles and the derivation of those outcomes from either IVF or ICSI generated embryos. The data includes the total productivity from fresh ETs, the first FET treatment cycle, any subsequent FETs in the timeframe (2011-2019) as well as the potential productivity (pregnancies, live births, and babies) from those embryos remaining in cryostorage. The statistical analysis compares the birth rates of ICSI-derived vs IVF-derived pregnancies.



4. Discussion

The data presented in this study examines an IVF-ICSI Split model for couples with unexplained infertility, where male factor is meticulously excluded and ART is conducted by a strict algorithm, a commitment to blastocyst culture, along with single embryo transfers and a high commitment to cryopreservation. From 242 treatment cycles, 3346 oocytes recovered (13.8 per OPU) were randomly allocated to IVF or ICSI and the fertilization rates standardized to the number of 2PNS arising from each group applying the metaphase II oocyte number identified for the ICSI group, as the denominator for both groups. Applying this adjusted formula for fertilization by IVF, we found the fertilization rates were significantly higher overall for ICSI (83.2% vs 65.4%; $p<0.0001$), being most pronounced for women under 40 years.

Nonetheless, we found that the resultant embryos from either IVF or ICSI had equivalent utilization rates for both fresh ETs and FETs so that pregnancy rates and ensuing live birth rates were equivalent in the first round of procedures. However, pursuant to the higher fertilization rates for ICSI, there were significantly higher numbers of ICSI-generated embryos cryopreserved and subsequent FET procedures (after the first round) showed higher live birth rates for the ICSI-generated embryos. This likely reflects the more diminished pool of embryos in cryostorage from the IVF arm, with the remainder comprising a smaller pool of lower quality embryos from which to select for second and subsequent FET procedures. On the contrary, the ICSI-generated pool of cryostored embryos comprised a significantly larger pool, with undoubtedly many high-quality embryos from which to select for further FET procedures.

Furthermore, our calculation of the potential live births from those embryos remaining in cryostorage at the completion of this study, indicates a further 214 live births potentially arising from the ICSI-generated embryos as opposed to 104 live births from the IVF-generated embryos. Our data is further reassuring as all the babies delivered from the live births in this study (198 babies comprising 89 babies from 86 IVF-generated births and 109 babies from 105 ICSI-generated births) were all healthy with no congenital abnormalities detected. This data might support the idea of encouraging ICSI fertilization for all ART cases, however we did show that a small proportion of women will demonstrate fertilization in the IVF-arm only (7.7%) without a single oocyte fertilizing from 5 injected in that same group. For this reason, PIVET encourages and practices a 90% ICSI fertilization rate and maintains the IVF-ICSI Split model for all IVF-naïve women categorized with unexplained infertility and where male factor has been clearly excluded following investigations which include a thorough clinical assessment, male urogenital ultrasound examination, semen profiling, ASAB and DNA fragmentation screening. The results from the IVF-ICSI mode carries high diagnostic predictive value and provides the lead for future ART procedures when required.

Although the study has a definable weakness in being a retrospective analysis of the IVF-ICSI Split mode of fertilization, it also has a strong positive feature in that the OCCs were allocated randomly by the embryologists, without reference to the various Consultant Clinicians who, although closely managing the procedural side of the women's management, were completely functioning outside the laboratory aspects which were decided by the embryology team, under the general direction of the Medical Director and the Laboratory Director.

Whilst the data from ICSI at PIVET clearly favors ICSI, this has not been a universal viewpoint [72,73] and the ASRM Practice committee has repeatedly advised against ICSI for non-male factor infertility [73,74]. Nonetheless, we have argued that our studies have applied a specific approach utilizing clear clinical criteria which includes a favorable FSH-dosing algorithm [7,45], a single embryo transfer policy of almost 100%, a blastocyst culture protocol for >90% of couples [45] and an ICSI protocol with injection of oocytes strictly 42±2 hours post HCG Trigger [68]. Furthermore, we have evaluated the male partner most carefully [75], with attention to clinical features as well as testing for anti-spermatozoal antibodies [8,76], DNA-fragmentation [40] and research with the hypo-osmotic swelling test, with the view to identifying the individual sperm with minimal DNA fragmentation [54]. We are aware that such intensive assessment of the male partner is not universally practiced [75]. However, we are aware that our protocols place our PIVET clinic in the highest quartile for live birth outcomes from initiated cycles in the Australian and New Zealand Assisted Reproduction Database [52]. It also means that our IVF±ICSI program removes any relevance of male age or semen parameters on clinical pregnancy and live birth outcomes [77]. Hence, we have no reservations about our data not being concordant with the past mainstream. We would respectfully suggest that other facilities trial PIVET protocols.

5. Conclusion

This study corroborates our view that most (90%) ART procedures should be conducted using the ICSI technique for fertilization – both for male factor and most non-male factor cases. However, the decision for using ICSI on cases of unexplained infertility should be decided by undertaking an IVF-ICSI Split method on all IVF-naïve women. This will enable the detection of those cases best suited to ICSI as well as avoiding cases of complete failed fertilization. It will also ensure that every couple has a reasonable chance of generating a live birth on their first ART treatment cycle.

Compliance with ethical standards

Acknowledgments

We are grateful for the close working relationship between the various Departments operating at PIVET Medical Centre, particularly the integration of nursing, laboratory and clinical areas for the daily Results Review meeting and the handling of patients within the Perth Day Surgery Centre for OPU and ET procedures. The nursing team at PIVET have

been fastidious in detailing clinical outcomes into registers, thereafter into the Filemaker database. Nurse Alison Pusey has been especially successful in tracking the outcome of each pregnancy, many of which resulted in deliveries in regional locations and sometimes overseas.

Disclosure of conflict of interest

The entire project has been funded internally at PIVET without any external or commercial contributions. The authors declare no conflicts of interest.

Statement of ethical approval

Reporting of the data was approved under Curtin University Human Ethics Committee approval no. RD-25–10 general approval for retrospective data analysis in 2010, updated in 2015, and again further updated in August 2020.

Statement of informed consent

PIVET is accredited with both the self-regulatory National Australian Reproductive Technology Committee (RTAC) as well as the Reproductive Technology Council (RTC) of Western Australia. Consent forms received approval under both regulatory bodies. The assay laboratory is accredited on an annual basis by the National Australian Testing Authority (NATA).

Author Contributions

The study was conceived by PIVET Medical Director JLY who established the data base at PIVET Medical Centre with the assistance of IT Consultant and data manager PMH. Lab Director JLC has directed embryologists NM, RG and JW in oocyte allocation for the project. All authors have assisted with the data analyses as well as the preparation of the Tables and Figures. The manuscript was written by JLY and each of the authors has read and agreed to its content.

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Author's Short Biography

Professor John Yovich MD PhD FRCOG FRANZCOG CREI graduated MBBS, now MD, from the University of Western Australia in 1970, progressing into Specialist O&G practice in 1976. Following laboratory research and clinical work over 4-years in London 1976-80 with Professor Ian Craft, John presented his MD, now PhD, thesis *"Human pregnancies achieved by in-vitro fertilization"* (UWA 1985). Established PIVET Medical Centre in 1981, generating WA's first IVF child in July 1982. Assisted many IVF clinics to establish worldwide, forming PIVET Malaysia in Kuala Lumpur with first IVF infant born May 1987, also assisted the first IVF infant for Greece delivered in May 1987 and established Cairns Fertility Clinic in 2008.