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Fertilization by ICSI generates a higher number of live births than IVF in a pioneer facility applying >90% single blastocyst-stage embryo transfers

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Abstract

Following earlier studies introducing an IVF-ICSI Split model on couples with unexplained infertility to avoid the scenario of unexplained failed or poor fertilization, PIVET has adopted a high ICSI rate approaching 90%, whereas the general rate among Australian facilities is around 60%. This observational study with retrospective data analysis reports on the IVF±ICSI procedures conducted over the period 2011 to 2019 with follow-up of ensuing pregnancies through 2020. Using autologous oocytes, 2343 women had 3434 IVF±ICSI cycles where 84.5% of women had 88.9% of initiated treatment cycles using ICSI and only 5.3% of women had 4.0% of cycles by IVF. The remaining 10.1% of women utilized the IVF-ICSI Split model for the remaining 7.2% of cycles. It was shown that oocyte fertilization rates were significantly higher for ICSI ($p<0.0001$), but not significant for women >40 years. The utilization rates of the ensuing embryos were ~45% across all ages with no significant differences across the ages, except for those small numbers of women ≥45 years who had a higher rate from IVF-generated embryos ($p<0.0002$). Pregnancy outcome were higher from ICSI-generated embryos across the age groups, being especially marked among the younger women <40 years ($p<0.0001$). Miscarriage rates were lowest for the IVF-generated pregnancies (overall 6.7% vs 22.8%, $p<0.0001$) but nevertheless the final live birth productivity rates per initiated treatment cycle remained higher from the ICSI-generated pregnancies (56.5% vs 46.3%; $p<0.0001$). Although this study does not meet the highest standards for EBM, it emanates from a pioneer facility with >40 years of published activity and which practices 90% blastocyst transfers in >90% SET cycles. The study supports a high ICSI rate of almost 90% and an IVF-ICSI Split rate of 10%.

Keywords: Assisted reproductive technology (ART); *In vitro* fertilization (IVF); Intracytoplasmic sperm injection (ICSI); IVF-ICSI Split; Miscarriage; Pregnancy productivity rate; Live birth productivity rate.

1. Introduction

Historically, the technique of in vitro fertilization (IVF) commenced in the United Kingdom as an activity supported under the national health service (NHS), thereafter moving into private practice facilities from 1980 [1,2]. Whilst the previous era focused on selecting young women with infertility related to tubal disease, the next era involved fee-paying couples with infertility due to a range of factors, with an increasing proportion having definable male factors [3,4], but many others having poorly explained [5,6] or entirely unexplained, factors [7]. With respect to male factor infertility, the early IVF methodology was seen to have limited success [8, 9, 10, 11] hence a number of laboratory-based methods were applied. These included specialized sperm preparations [12], pentoxifylline enhancement of both sperm motility and function [13,14], and micromanipulation methods such as partial zona dissection (PZD) and sub-zonal insemination (SUZI) [15], culminating in the highly effective intra-cytoplasmic sperm injection (ICSI) technique, reported in 1992 [16]. The ICSI procedure was initially applied for male factor due to suboptimal semen profiles, but soon expanded to

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cases with anti-spermatozoal antibodies in either the male or female partner, as well as for cases requiring surgical sperm recovery [17]. Subsequently, albeit as a controversial progression, ICSI has been applied to non-male factors including unexplained infertility, followed by "precious" scenarios such as older women with few oocytes in order to avoid the risk of failed fertilization (18).

Team leader and senior author (JLY) commenced IVF practice assisting Professor Ian Logan Craft (ILC) to establish his pioneer facility in London across the period 1976-1980. Together JLY and ILC recognized at an early stage that poor fertilization was often a consequence of some deficient "sperm factor", despite a normal semen analysis profile hence other laboratory tests have been explored including a test for the acrosome reaction [19], research into sperm chromatin structure [20] and DNA fragmentation along with excess reactive oxygen species (ROS) in semen and methods to identify the optimal spermatozoon with minimal DNA fragmentation [21], one which could be ideal for ICSI. However, laboratory tests evolving from these studies have proven of limited clinical value [22] and led to PIVET's current clinical practice of applying an IVF-ICSI Split model for IVF-naïve cases having unexplained infertility [23]. This enabled both a diagnostic test for the fertilization potential of a semen sample (from the IVF arm), as well as providing a therapeutic benefit at the first IVF procedure (from the ICSI arm) and avoiding the clinical distress associated with poor, or zero fertilization in the couple's first treatment cycle. This preamble and evolution of the ICSI technique accords with the pioneers of the procedure [18].

The most recent annual ANZARD (Assisted Reproductive Technology in Australia and New Zealand) report details the clinical outcomes for all IVF procedures undertaken during 2018 with live births completed by October 2019 [24]. This 2020 ANZARD report shows that a single embryo transfer (SET) was undertaken in 91% of women across all ages and the majority of those SET procedures involved blastocysts (94%). Consequently, multiple births are now recorded in only 3.2% of pregnancies whilst live birth rates have actually increased to 27.3% across all ages for autologous cycles reaching embryo transfer. Over the 5-year period 2014 to 2018, ICSI rates have ranged from 63.8% to 60.2% of IVF cycles, the most recent reduction probably arising from an Australian report which concluded that ICSI does not increase the cumulative live birth rate in non-male factor infertility [25]. However, our experience at PIVET in Western Australia does not accord with that of the Victorian study, neither in its findings regarding fertilization rates nor that of the similar live birth numbers to IVF. Furthermore, we have not found any increase in adverse peri-natal outcomes from the ICSI-generated pregnancies at PIVET and refute the comment. We recognize numerous problems with the Victorian study, many of which have been identified by those authors, one of which is that it draws data from numerous IVF facilities with diverse clinical practices and variable success rates which can be deduced from a freely accessible website [26] which was not available at the time the study was published.

We have therefore undertaken our study from a single IVF facility which has a unique, but well described clinical and laboratory practice where the success rates are known to be high and freely accessible. The study presented here examines both the laboratory and clinical outcomes of practicing a higher ICSI rate approaching 90% of cycles reaching oocyte pick-up (OPU), as proposed in an earlier study from PIVET [27].

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. 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 ILC as stated in the Introduction. Pregnancies from IVF were established in the latter half of 1981 and the first live birth occurred in July 1982. 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 [28]. 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 [29] with validation of an optimized pregnancy productivity rate, and almost complete avoidance of ovarian hyperstimulation syndrome [30]. In almost 90% of OPU's oocyte numbers range from 8-12 for standard cases, adapted for a long-acting FSH product [31] and adjusted for lower numbers suited to a Low-cost, minimal intervention program [32].

Table 1 Displays one of the PIVET FSH-dosing algorithms which have been utilized throughout the study period. The algorithms enable small FSH dosing increments as low as 8.3 IU for the Puregon pen as shown here (red sector) and 12.5 IU for the Gonal-f pen (orange sector) and biosimilars. From 300 IU FSH the dosing increments are ≥25 IU FSH hence this algorithm applies for both pens as well as the long-acting FSH preparation Elonva (green sector).

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 [7,28]. 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 [33]. Under an ANZARD initiative, the results from all participating IVF units in Australia and New Zealand can now be viewed [26] and reveals that PIVET's IVF program generates live birth productivity rates [34] in the highest quartile across all age groups of women. These high live birth productivity rates are achieved whilst recording multiple pregnancies in <3% of births (being twins only) and complications such as severe ovarian hyperstimulation syndrome (OHSS) requiring hospitalization occurs 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.

2.2.1. Male infertility factors

Male infertility is diagnosed according to the semen parameters defined by the WHO laboratory manual of 2010 [35] 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 [23]. Male factor infertility also includes those cases requiring surgical sperm retrieval [17] 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 past 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 [37], 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, 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.

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 [38]. 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 [39] or only a single oocyte is retrieved [40]. 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 [41] 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 and these can mostly be resolved by ICSI.

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". 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 [42]. 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 [43], 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 (>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 [23]. 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.

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 [27] and indicated by others [44]. 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 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 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 [45].

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 [27].

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. 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 [28]. 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 [28]. High-risk pregnancies and those with threatened miscarriage were offered medroxyprogesterone acetate during the first trimester, continuing to 35-weeks gestation where pre-term delivery has occurred previously. We have recently published beneficial outcomes from this strategy [46].

2.5. 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 xDiagram version 5.4 (created by Vu Tien Thinh), thereafter exported as a Tiff File. 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 $p<0.01$ 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 Tiff files.

3. Results

Across the period 2011 to 2019, 2443 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 one's self. This included the idea of cryopreservation of embryos and maximizing the productivity rate from a single initiated IVF cycle as reported in this study.

From the Flow sheet (Figure1) it can be seen that the majority of treatments were undertaken utilising 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 237 women (10.1%) initiated 287 (7.2%) treatment cycles where ≥4 oocytes were randomly allocated to either IVF or ICSI insemination. The number of women and their IVF±ICSI cycles are shown in specific display boxes according to age categories.

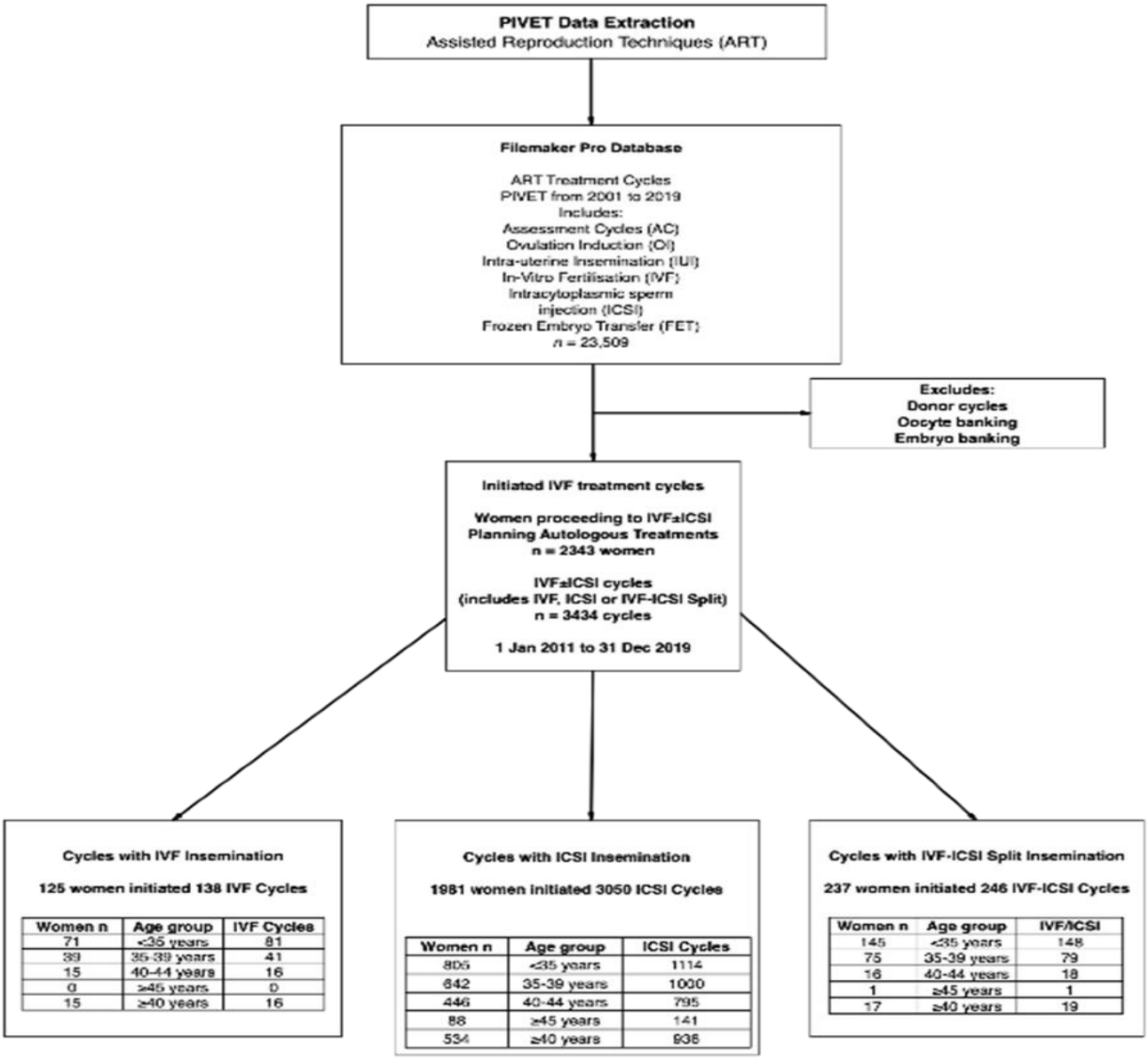

Figure 1 Flowsheet depicting the derivation of 3434 IVF±ICSI treatment cycles from 2343 women across the period 1 January 2011 until 31 December 2019 with pregnancy outcomes tracked throughout 2020. The treatments are subcategorized according to the insemination process; being IVF, ICSI or an IVF-ICSI Split mode.

The laboratory data including oocyte numbers retrieved at OPU and the insemination outcomes (IVF vs ICSI) are depicted in Table 2. It is shown that the 2PN (pronuclear stage) which identified normal fertilization was identified in 18383 of the 25159 oocytes inseminated (73.1%) overall from a total 34126 oocytes collected at OPU. This means 15.1% 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 1906 2PNs arising from 3186 having IVF insemination (59.8%) to 16022 of 21973 oocytes (72.9%) having ICSI insemination. 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 <40 years (Figure 2). The apparent benefit for ICSI among the women ≥40 years proved not significant, even for those women ≥45 years albeit numbers were quite small in this range of advanced female age.

The utilization rates of those embryos arising from the fertilized oocytes are shown in Table 2 which denotes them as either IVF or ICSI-derived. A proportion of the embryos were transferred in fresh cycles (total n=3270) whilst a larger proportion were cryopreserved (total n=5667). During the 9-year study period 2011-2019, a proportion of these were transferred as FET cycles following a variable period of cryopreservation, some of which occurred prior to 2011. This

meant that 2339 embryos which were IVF-derived had FET procedures and 5718 embryos which were ICSI-derived had FET procedures. Overall fresh ETs and FETs combined totalled 8057 procedures utilizing 8937 embryos indicating that 90.2% of procedures were conducted as SETs over the entire 9-year period. The embryo utilization rates across the age groups are shown in Figure 3 where it can be seen that the rates were ~45% with no significant differences across the groups <45 years. However, in the oldest group of women ≥45 years there was a significantly higher utilization for IVF-derived embryos ($p<0.0002$) 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.

Table 2 Details the embryology data arising from the 3434 cycles which reached the stage of OPU. From 34,126 oocytes, the fertilization outcomes from either IVF or ICSI are shown (see Figure 2) as well as the utilization of the embryos arising from those fertilizations (see Figure 3).

IVF±ICSI Cycles 2011-2019	Embryology Data				
Women's Age Groups years	**<35**	**35-39**	**40-44**	**≥45**	**Total**
Initiated IVF±ICSI Cycles	1442	1228	931	166	3434
- Cycles for IVF insemination	81	41	16	0	138
- Cycles for ICSI insemination	1114	1000	795	141	3050
- Cycles for IVF-ICSI Split	148	79	18	1	246
Women represented	1021	756	477	89	2343
Cancelled cycles *n*	3	8	9	0	20
OPU cycles n	1340	1112	820	142	3414
Oocytes collected *n*	15914	11433	6025	754	34126
Oocytes / OPU	11.9	10.3	7.4	5.3	10.0
- Oocytes with IVF insem.	1888	1021	275	2	3186
- Oocytes with ICSI insem.	9890	7357	4156	570	21973
Total # 2PNs arising	8684	6171	3119	409	18383
- from IVF	1088	639	178	1	1906
- from ICSI	7447	5362	2812	401	16022
Freeze-all embryo cycles	161	153	117	26	457
Fresh Embryo Transfer Cycles	1131	912	640	93	2776
Embryos transferred fresh *n*	1183	1061	896	130	3270
- IVF derived embryos ET	131	67	20	1	219
- ICSI derived embryos ET	1030	974	847	127	2978
Embryos cryopreserved	3103	1840	635	89	5667
- IVF derived embryos	411	225	43	0	679
- ICSI derived embryos	2635	1578	559	88	4860
Frozen Embryo Transfer Cycles	1169	783	355	46	2353
Embryos transferred frozen *n*	1178	820	395	55	2448
- IVF derived embryos FET	1098	786	393	62	2339
- ICSI derived embryos FET	2361	1881	1291	185	5718
Total Transfers ETs + FETs	3459	2667	1684	247	8057
Total Embryos Utilized	4286	2901	1531	219	8937

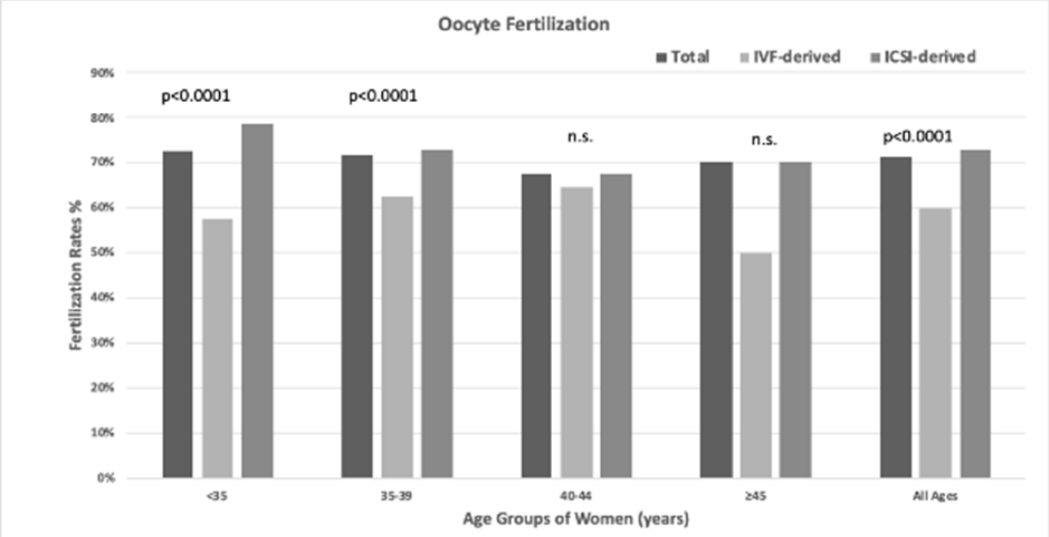

Figure 2 Displays the fertilization rates for oocytes derived from women undergoing IVF±ICSI procedures according to the insemination mode (IVF or ICSI). The fertilization rates were significantly higher across all the women's age groups, proving highly significant for the younger women <40 years.

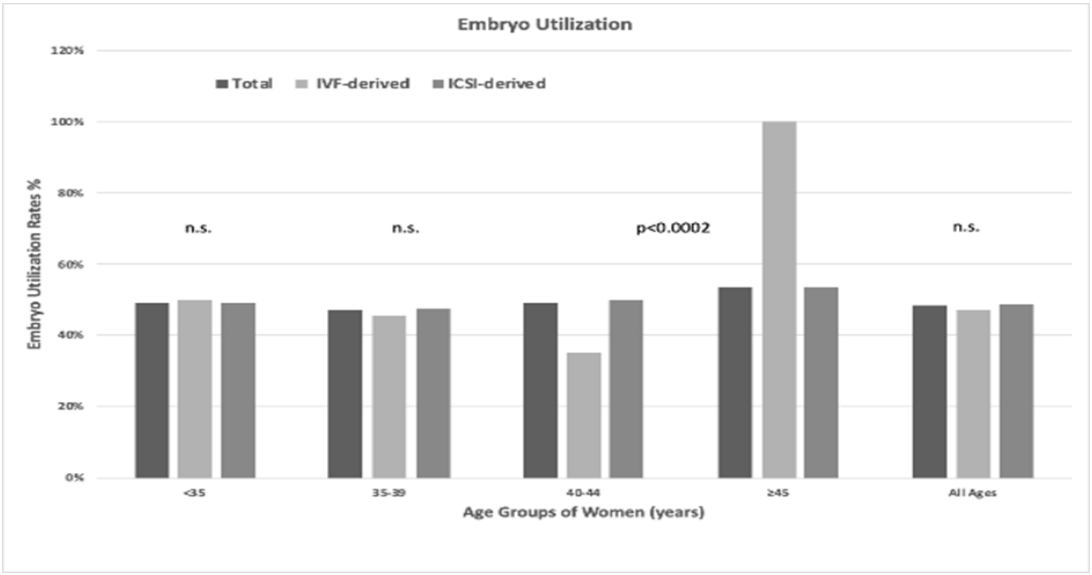

Figure 3 Displays the embryo utilization rates for those fertilized oocytes which resulted in a fresh embryo transfer procedure or a subsequent frozen embryo transfer following a period of cryopreservation. Across all the age groups there were no significant differences between IVF-generated embryos nor ICSI-generated embryos, indicating equal potential for utilization based on morphological criteria. The aberrant significance in favour of IVF for women aged ≥45-years may be a reflection of small data for this sub-group or other confounders (see text).

The clinical outcomes data is shown in Table 3, where the pregnancy productivity rates (following fresh ETs and FETs) for initiated cycles is seen to be 46.2% across all ages, ranging from 68.5% for young women <35 years, reducing sequentially to 45.6% for those aged 35-39 years, thereafter 18.9% for women aged 40-44 years and a low 10.2% for those women aged ≥45 years.

Table 3 Details the clinical data arising from the transferred embryos including the pregnancy, miscarriage and live birth rates arising from fresh embryo transfers as well as from the frozen embryo transfers. This enables the compilation of pregnancy and live birth productivity rates as shown in Figures 4 and 6, respectively. The data is presented for both IVF and ICSI-generated pregnancies as well as the combined totals.

IVF±ICSI Cycles 2011-2019	Clinical Outcomes Data				
Women's Age Groups years	**<35**	**35-39**	**40-44**	**≥45**	**Total**
Initiated IVF±ICSI Cycles	1442	1228	931	166	3434
- Cycles for IVF insem	81	41	16	0	138
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- Cycles for IVF-ICSI Split	148	79	18	1	246
Women represented	1021	756	477	89	2343
Cancelled cycles *n*	3	8	9	0	20
OPU cycles n	1340	1112	820	142	3414
Oocytes collected *n*	15914	11433	6025	754	34126
Total ET + FET cycles n	1098	786	393	62	2339
Pregnancies *total fresh n*	449	236	90	8	783
- from IVF fresh ETs	67	25	9	0	101
- from ICSI fresh ETs	382	211	81	8	682
Pregnancies *total frozen n*	539	324	86	9	958
- from IVF FETs	105	42	4	3	154
- from ICSI FETs	434	282	82	6	804
Pregnancy Productivity ETs + FETs total n	988	560	176	17	1741
- Preg Prod from IVF	172	67	13	3	255
- Preg Prod from ICSI	816	493	163	14	1486
- Preg Prod % / initiated cycle	68.5%	45.6%	18.9%	10.2%	46.2%
- Preg Prod % from IVF	27.5%	12.3%	9.0%	3.3%	15.2%
- Preg Prod % from ICSI	100%	72.3%	31.6%	18.7%	71.1%
Statistics	P<0.0001	P<0.0001	P<0.0001	P<0.002	P<0.0001
- Preg Prod total / OPU	68.7%	45.9%	19.1%	10.2%	46.5%
- Pre Prod total / ET Cycle	90.0%	71.3%	44.8%	27.4%	74.4%
Pregnancy losses n	169	115	65	7	356
Miscarriage rate %	17.1%	20.5%	36.9%	41.2%	20.5%
Live births *total fresh n*	383	184	48	5	620
- from IVF fresh ETs	57	24	5	0	86
- from ICSI fresh ETs	326	160	43	5	534
Live births *total frozen n*	794	425	108	10	1337
- from IVF FETs	425	207	53	5	690
- from ICSI FETs	369	218	55	5	647
Live birth Productivity ETs + FETs total n	1177	609	156	15	1957
- L/B Prod from IVF	482	231	58	5	776
- L/B Prod from ICSI	695	378	98	10	1181
- L/B Prod % total / init	81.6%	49.6%	16.8%	9.0%	52.0%
- % IVF L/B Prod / init	77.0%	42.3%	14.0%	5.5%	46.3%
- % ICSI L/B Prod / init	85.2%	55.4%	19.0%	13.3%	56.5%
- Statistics	P<0.0001	P<0.0001	P<0.05	n.s.	P<0.0001
- L/B % per OPU cycle	81.8%	34.8%	11.7%	6.0%	35.7%
- L/B % per ET cycle	107.2%	54.1%	27.5%	16.1%	57.2%

These rates embraced significant differences across the entire age range between IVF-derived pregnancies (15.2%) and pregnancies from ICSI-derived embryos (71.1%; p<0.0001) as shown in Figure 4 and were especially marked among the younger age-groups.

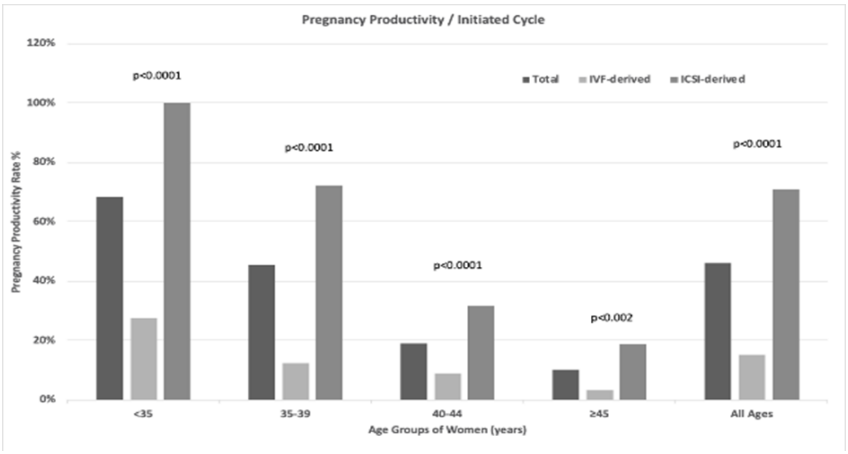

Figure 4 Displays the pregnancy rates following the transfer of either fresh or frozen embryos as a productivity rate from a single initiated IVF±ICSI treatment cycle. Across all the age ranges of the women, the ICSI-generated embryos resulted in a significantly higher pregnancy productivity rate from a single initiated treatment.

The data on pregnancy losses is shown in Table 4 where the rates ranged from a low of 17.1% for young women aged <35 years, rising sequentially to 20.5% for women aged 35-39 years, thereafter 36.9% for women aged 40-44 years and 41.2% for women aged 45 years. Overall, 20.5% of pregnancies failed to progress beyond 20-weeks' gestation, most being lost in the first trimester from 6-weeks to 10-weeks' gestation.

Table 4 Details the pregnancy losses (to 20-weeks) of the pregnancies shown in Table 3 with analyses according to the woman's age group as well as according to the mode as an IVF or ICSI-generated pregnancy. Miscarriage was significantly higher among ICSI-generated pregnancies, especially for younger women ≤40 years.

IVF±ICSI Cycles 2011-2019	Pregnancy Loss Data				
Women's Age Groups years	**<35**	**35-39**	**40-44**	**≥45**	**Total**
Initiated IVF±ICSI Cycles	1442	1228	931	166	3434
- Cycles for IVF insem	81	41	16	0	138
- Cycles for ICSI insem	1114	1000	795	141	3050
- Cycles for IVF-ICSI Split	148	79	18	1	246
Women represented	1021	756	477	89	2343
Cancelled cycles *n*	3	8	9	0	20
OPU cycles n	1340	1112	820	142	3414
Oocytes collected *n*	15914	11433	6025	754	34126
Total ET + FET cycles n	1098	786	393	62	2339
Pregnancies total fresh n	449	236	90	8	783
- from IVF fresh ETs	67	25	9	0	101
- from ICSI fresh ETs	382	211	81	8	682
Pregnancies total frozen n	539	324	86	9	958
- from IVF FETs	105	42	4	3	154
- from ICSI FETs	434	282	82	6	804
Preg Prod ETs + FETs total n	988	560	176	17	1741
- Preg Prod from IVF	172	67	13	3	255
- Preg Prod from ICSI	816	493	163	14	1486
Pregnancy losses total n	169	115	65	7	356
- IVF-derived pregnancies	13	2	2	0	17
- ICSI-derived pregnancies	156	113	63	7	339
Miscarriage rate %	17.1%	20.5%	36.9%	41.2%	20.5%
- IVF-derived pregnancies	7.6%	3.0%	15.4%	0.0%	6.7%
- ICSI-derived pregnancies	19.1%	22.9%	38.7%	50.0%	22.8%
Statistical differences IVF vs ICSI	P<0.0001	P<0.0001	n.s.	n.s	P<0.0001

As depicted in Figure 5, there were marked differences in the pregnancy losses between IVF-derived vs ICSI-derived pregnancies, in favor of IVF-generated embryos. Overall, the miscarriage rate was a low 6.7% among the IVF-generated

pregnancies, as opposed to 22.8% for ICSI-generated pregnancies (p<0.0001) and these differences prevailed across the age groups, being most significantly marked among the younger women aged ≤40 years.

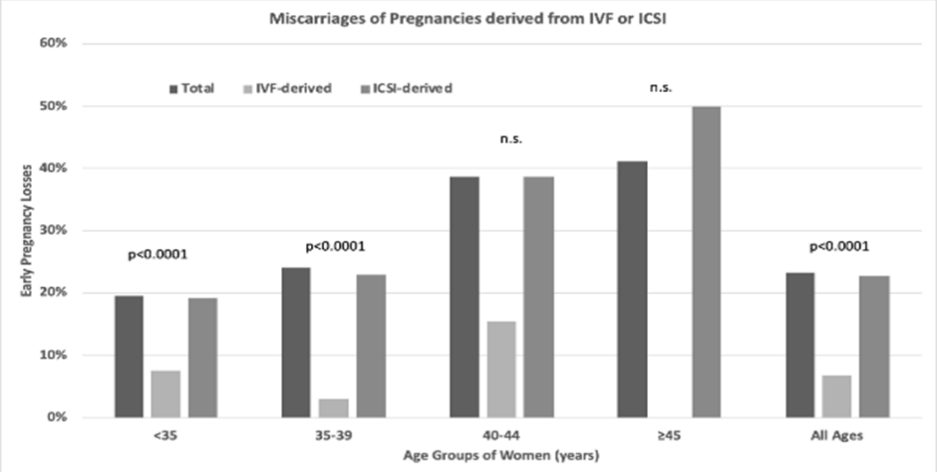

Figure 5 Displays the pregnancy losses arising from those implantations diagnosed as "clinical pregnancies" but which failed to progress to 20-weeks' gestation. All losses including ectopic gestations, pregnancies of unknown location and demised intra-uterine gestations (with or without curettage) are included under the heading of "miscarriages". There was a significantly higher likelihood of miscarriage from an ICSI-generated pregnancy, especially among younger women <40-years.

The livebirth productivity rates per initiated cycles was also shown in Table 3 indicating overall rates ranging from 81.6% for young women <35 years, falling sequentially to 49.6% for women aged 35-39 years, thereafter 16.8% for women aged 40-44 years and a low of 9.0% for the oldest women ≥45 years. As depicted in Figure 6, these rates embraced significant differences between pregnancies from IVF-derived embryos (58.0%) and those from ICSI-derived embryos (88.3%; p<0.0001) across the entire age range (and especially marked among the younger age-groups <40 years. Despite the significantly higher miscarriage rates among the ICSI-generated pregnancies (Figure 5), there were still significantly more live births overall per initiated treatment cycle from the ICSI-generated embryos (56.5% vs 46.3%; p<0.0001).

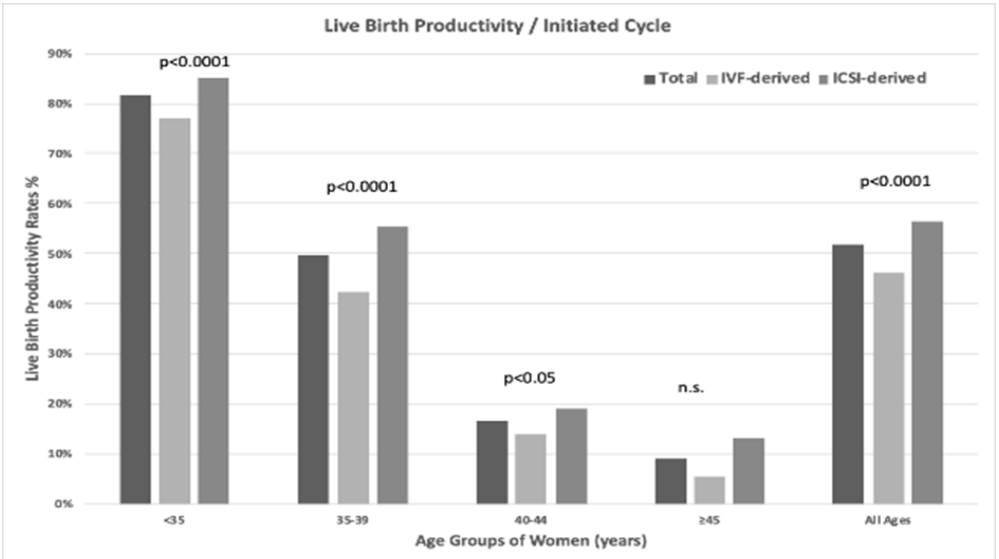

Figure 6 Displays those pregnancies which resulted in a live birth outcome arising from a single initiated IVF±ICSI treatment cycle. Despite the higher miscarriage rate among ICSI-generated pregnancies, the live birth productivity rate remains significantly higher for the ICSI group. The pregnancies are derived from both fresh and frozen embryos as a productivity rate for an initiated treatment cycle. Twin gestations are classified as a single birth.

The vast majority of pregnancies were singleton resulting in single infant livebirths at term (≥37 weeks' gestation). Although not presented here, stillbirths were rare, preterm delivery rates were not raised above natural background levels, both perinatal morbidity and mortality levels were not elevated, and fetal abnormalities were rare. There were no differences detected between IVF-generated and ICSI-generated babies.

4. Discussion

Whilst the prevailing view indicates that ICSI should be reserved for those couples with an identifiable male factor cause for their infertility [47,48,49], PIVET has pursued the idea that the IVF method of insemination should be reserved for those cases where a benefit has been demonstrated. Such benefit may have been shown from a previous IVF procedure where ≥50% of oocytes recovered had developed into good quality embryos. In modern day this means blastocyst development of sufficient quality enabling both a fresh SET as well as at least one blastocyst cryopreserved for future FET where 10±2 oocytes had been recovered. In the absence of a more reliable predictive laboratory test than either semen analysis or sperm DNA fragmentation testing, PIVET's approach has been to identify the fertilizing potential of the male partner by the IVF-ICSI Split procedure in IVF-naïve cases. Following our earlier reported studies defining a range of non-male factor criteria for ICSI [27], PIVET has maintained its current approach to IVF management and the study reported here from a 9-year period was intended to evaluate this. For those requiring the highest level of evidence-based medicine (EBM) reporting, a proposed large prospective RCT from China is awaited [50]. Meanwhile, a retrospective analysis on 144,018 IVF±ICSI cases from the United Kingdom over the period 2003 to 2007, actually showed an OR for live birth of 1.7 in favor of ICSI [51].

The findings in our study provides some mixed messages, but overall supports PIVET's approach that an overall ICSI rate of ~85% which includes a number of non-male factor scenarios, optimises the clinical outcomes for IVF management and results in more live babies arising from the overall IVF program. Furthermore, adding the IVF-ICSI Split model for new cases (IVF-naïve women) avoids the situation of unexpected poor fertilization (15% chance) including failed fertilization (5% chance). This brings the overall ICSI utilization close to our earlier suggestion of 90% for optimal clinical benefit [27]. Furthermore, it also avoids using the IVF treatment as a diagnostic test where couples may face a distressing clinical scenario with adverse financial consequences if being managed in the private sector. The achievement of at least a proportion of fertilizations (from the ICSI arm) provides some relief from both clinical and financial stresses as well as availing the couple the opportunity to achieve a live healthy infant. This is particularly relevant among the younger groups of women with age <40 years. Although heavily criticized for a number of deficiencies, two Cochrane studies of 2000 and 2003 have actually supported ICSI for borderline sperm disorders as well as normozoospermic cases [52,53]. The most recent reported study also supports the IVF-ICSI Split model showing a higher blastocyst development rate for those oocytes inseminated by ICSI [54].

Although our data shows a significantly higher fertilization rate as well as pregnancy rates arising from ICSI-generated embryos, the finding of a significantly higher miscarriage rate from ICSI-generated pregnancies accords with the view that ICSI is likely to cause a higher rate of abnormal fertilizations given the numerous physiological processes by-passed by the ICSI methodology. The best studies in this respect arise from the research group headed by Gerald Schatten, which has been studying IVF vs ICSI on primates from the Oregon National Primate Research Centre [55]. The Oregon group have recently reported that although the non-primate models differ in fertilization processes, particularly related to events after sperm-oolemma binding, they do show significant differences in microtubule spindle formation and interactions at the cortex, as well as orientation differences of the male and female pronucleus between the IVF and ICSI fertilized oocytes. With the further knowledge that the centrosomes of the developing embryo are purely of paternal origin, and house the microtubule-organizing center [56], embryologists have been reticent to endorse the universal notion of ICSI replacing IVF. However, notwithstanding these observational differences, time has been reassuring in revealing no increase in fetal and developmental abnormalities from the ICSI process. The reported difference of 4.0% for IVF and 7.1% for ICSI children relates to confounders underlying the male infertility cases, a difference not seen in outcomes from ICSI performed for non-male factors such as donor oocytes, low oocyte numbers and unexplained infertility [57]. However, the notion of ICSI for all is not universally required and some allowance should be given for individual preferences, particularly where some fertilization was obtained from IVF in the ICSI-Split insemination model. So too, IVF may be preferred, as our data also suggests, for the potentially fragile single oocyte retrieved from women of advanced age >40 years). By preserving this notion of IVF where "proven" to be equivalent to ICSI fertilization, the ICSI rate will be near 90%. Our emergent data indicates that this accords with happier patients, less distress to the IVF team and no recognizable adverse outcomes.

For those IVF clinics who prefer to avoid strategies for IVF, ICSI or IVF-ICSI Split insemination, a 100% ICSI rate can be endorsed from the current viewpoint, where the ICSI operator has sufficient expertise to minimize oocyte damage (usually <2.5% in oocytes with MII-spindle demonstrated by the PolScope oocyte imaging system (Oosight™); but can

be ≥5% when the spindle is not visible or specific imaging is not applied. Destruction of an oocyte by ICSI can be a distressing problem for the patient, the embryologist and the clinician when this was the only oocyte recovered at OPU. With respect to procedural timings and operators undertaking ICSI, it is apparent that practice is quite varied with many modern operators having little understanding of the evolutionary developments for optimal fertilization of oocytes [58]. It therefore appears at this stage that some cases will indeed require management by IVF for best practice, so the current position of comfort is an ICSI rate of ~90%, as practiced at PIVET.

4.1. Limitations of this study

As an observational study with retrospective analysis of non-randomized data, (apart from the oocytes for the IVF-ICSI Split model), this study does not even approach the higher standards of EBM requirements. Nonetheless, we believe it serves an important contribution in the evolution of IVF best practice, given that the data emerges from a pioneer IVF clinic which has had an open publication record over more than 40 years to explain all of its developmental processes. We do acknowledge that the higher miscarriage rate for ICSI-generated pregnancies is a continuing matter of concern, requiring further fundamental scientific research. Furthermore, we accept that the IVF-ICSI Split model may be biased against IVF as those oocytes allocated to ICSI have the cumulus layer stripped to identify the mature MII oocyte prior to injection. Notwithstanding these observations, the over-riding clinical benefits depicted in this study justifies an ICSI rate above the current general level of 60% to one approaching 90%.

5. Conclusion

This study involved 2343 women having 3434 IVF±ICSI cycles and included a group (10.1%) having an IVF-ICSI Split model. The outcomes of IVF-generated embryos and ICSI-generated embryos were then compared in an observational study with retrospective data analysis. The ICSI procedure generated higher fertilization rates for those younger women <40 years however the resulting embryos showed equivalent utilization rates, albeit that the small group of women aged ≥45 years, had higher utilization from IVF-generated embryos. Pregnancy productivity rates (from both fresh and frozen ETs per initiated cycle) were significantly higher from ICSI-generated embryos, more marked for the younger women. However, ICSI-generated pregnancies had a significantly higher miscarriage rate than the IVF-generated pregnancies. Nonetheless, the live birth productivity rates remained significantly higher from the ICSI-generated pregnancies. Although IVF may have some benefits for older women >40 years, PIVET's approach with an overall ICSI rate approaching 90% and an IVF-ICSI rate of 10% appears well supported from this study.

Compliance with ethical standards

Acknowledgments

The authorship listing includes those members of the PIVET Laboratory team exploring the ICSI fertilization process and the associated embryology outcomes for the 10-year duration of this study. We are also grateful for the predecessors who established those important laboratory methods referenced here including James D Stanger, Jeanne M Yovich, Stephen M Junk, James M Cummins, Anne M Jequier, Steven J Yovich, Rohini W Edirisinghe, Long Vo, Phillip L Matson and Kevin N Keane.

Disclosure of conflict of interest

The entire project has been funded internally at PIVET without any external or commercial contributions. The patients involved paid the extra fee for ICSI procedures after providing informed consent. The authors declare no conflict of interest, other than receiving standard salaries and consulting fees as part of their employment with PIVET Medical Centre.

Statement of ethical approval

Reporting of this 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 for 5 years.

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). All the IVF-related processes mentioned in this article were provided under informed consent with women

providing written approval, witnessed by an independent person. The specific information and consent form for ICSI is embraced in a composite booklet comprising regularly updated patient-directed information as well as a specific area for signing and witnessing.

Author Contributions

The study was conceived by PIVET Medical Director JLY (MD, PhD, FRCOG, FRANZCOG, CREI) who established the data base at PIVET Medical Centre with the assistance of IT Consultant and data manager PMH (BAppSc). Embryologists JLC (Laboratory Director with BSc. & Master of Clinical Embryology), NM (BSc. Hons), JW (BSc. & Master of Embryology) and RW (BSc.) have each achieved competency certification in ICSI and have been involved in many research studies during their long-standing employment at PIVET. They have each been intimately involved in the ICSI-related studies reported here. The manuscript was written by JLY and each of the authors have read it and agreed to its content.

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Author's short biography

	**Professor Dr. John Yovich** MD PhD FRCOG FRANZCOG CREI graduated, 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 PhD thesis *"Human pregnancies achieved by in-vitro fertilization"* (UWA 1985). He established PIVET Medical Centre in 1981, generating WA's first IVF child in July 1982. John has assisted many IVF clinics to establish worldwide during the 1980's and, at age 75 years, maintains a full-time clinical program for fertility-related gynecological disorders as well as IVF. His academic work is now conducted through the Department of Pharmacy and Biomedical Sciences at Curtin University.