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Applying growth hormone as an adjuvant to correct poor prognosis outcomes in IVF: Study 2 compares dehydroepiandrosterone

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Abstract

This retrospective study examines the influence of recombinant growth hormone (rGH) and dehydroepiandrosterone (DHEA) adjuvants on oocyte numbers, embryo utilization and live births arising from 3637 autologous IVF±ICSI treatment cycles undertaken on 2376 women across ten years (2011-2020) within a pioneer Australian facility. Despite using an FSH-dosing algorithm enabling maximal doses up to 450 IU for women with reduced ovarian reserve, younger women had significantly higher mean numbers of oocytes recovered than older women ranging from 11.1 for women <35 years to 9.4 for women aged 35-39 years reducing to 6.5 for women aged 40-44 years and 4.1 for those aged ≥45 years ($p<0.0001$). Overall, the embryo utilization rate was 48.5% and live birth productivity rate was 35.4 % across all ages and neither rGH nor DHEA showed any benefit on these rates, in fact, those women with nil adjuvants showed the highest live birth rate per initiated cycle (44.94% overall: $p<0.0001$, and 55.2% for the youngest group: $p<0.001$). Embryo utilization was increased by rGH in those women aged 40-44 years who had low ovarian reserve ($p<0.0001$), but this benefit did not translate into any improvement in the live birth rate, in fact those women who did not use adjuvants had the highest overall birth rate ($p<0.0001$). Similarly, other factors known to cause a poor prognosis, including low IGF-1 profile, recurrent implantation failure, and low oocyte numbers at OPU, showed no improvement in embryo utilization nor in live births from the adjuvants. The relevance of embryo quality was examined on 1135 women whose residual embryos after a single fresh-embryo transfer failed to develop to a suitable grade for cryopreservation. From 1727 cycles such women often displayed an improved embryo utilization rate with both rGH, and with DHEA or combined rGH+DHEA. Even so, live birth rates were not improved by either of the adjuvants excepting young women <35 years using rGH without DHEA ($p<0.05$). Examining poor prognosis sub-groups, indicated both rGH and DHEA or combined rGH+DHEA consistently improved embryo utilization in those women with low ovarian reserve ($p<0.0001$), or those with low IGF-1 levels ($p<0.0001$) or with recurrent implantation failure ($p<0.02$). All the poor-prognosis sub-groups showed low live birth rates and, notwithstanding the improvements in embryo utilization, the live birth rates were not significantly improved by the adjuvants, albeit the rates were closer to the nil adjuvant groups (not significantly different).

Keywords: *In vitro* fertilization [IVF]; Intracytoplasmic sperm injection [ICSI]; Adjuvants; Recombinant growth hormone [rGH]; Dehydroepiandrosterone [DHEA]; Live birth productivity rate [LBPR]

1. Introduction

Although assisted reproductive technology (ART) has earnt a well-respected position in modern medicine with its current widespread application generating more than 10 million offspring since the first child born in 1978, in truth the technology has a poor prognosis for a large proportion of patients [1,2]. Apart from the main limiting factor, that of

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advanced female age ≥40 years, studies have shown several other specific variables which can limit the prognosis, namely the woman's ovarian reserve [3], which itself is attendant upon the antral follicle count (AFC) and the serum anti-Mullerian hormone [AMH] level [4], as well as the woman's IGF serum profile represented by Insulin growth factor-1 [IGF-1], Insulin growth factor binding protein-3 [IGFBP-3] and the IGF Ratio, being IGFBP-3/IGF-1 [5]. Specific studies conducted at our PIVET facility have excluded variables such as the woman's stature, her body weight, or her body mass index [BMI] as having any relevance to ART treatment outcomes [6], albeit that weight and BMI have well-known influence on fertility and can be part of the underlying reason for women attending ART facilities. Furthermore, following the introduction of the intracytoplasmic sperm injection [ICSI] methodology in 1992, most male factor cases pose no limitation to ART outcomes [7]. Even the age of the male partner has minimal influence on outcomes when such studies consider all the relevant female factors into the analyses [8].

Given the recently defined confounding variables underlying the poor-prognosis outcomes in current day [3,5,9], several adjuvants have been introduced into clinical practice with the view of improving ART outcomes. At PIVET we have been exploring three of these, namely growth hormone [10-12], dehydroepiandrosterone [DHEA) [13,14] and melatonin [15]. Following an encouraging report in 2005 on the use of recombinant manufactured growth hormone; rGH as an adjuvant for older women undergoing assisted reproduction [16], our PIVET team has reported on its use since 2010. From nearly 50 clinical reports on women with various poor prognosis factors, mostly involving retrospective studies, it appears that embryo utilization is increased in the vast majority [17] but improvement in live birth rates is reported in under 50% and those studies all display design weaknesses with problematic confounding variables [18,19]. To minimize the latter, we present a 10-year study period from our pioneer Australian facility practicing a stable protocol where blastocyst culture, single embryo transfers and an advanced cryopreservation program underlie a clinical regimen dictated by a validated Follicle Stimulating Hormone [FSH]-dosing algorithm which generated 10±2 oocytes for most of the women. This is the second study from this period, the first reported on rGH ± melatonin [15] whilst this study from the same period reports on rGH±DHEA.

2. Material and methods

2.1. Study setting

Following a 4-year period of training in London during the years 1976-1980, lead author JLY established the PIVET program for ART in Perth, Australia in 1981 [1,2], initially recording all treatment cycles and clinical outcomes in hand-written registers. From 2001, all ART treatments, numbering 23,509 have been comprehensively recorded in an internal validated data base using Filemaker Pro as well as providing data to ANZARD which publishes an annual report, available to the public [20]. Recently, as an Australian Government initiative, ANZARD also provides data to the YourIVFSuccess website, enabling an open disclosure of results from each of 90 participating IVF Clinics around Australia [21]. Since January 2011, the ART program at PIVET has been characterized by three important developments, firstly, encouraging amenable ART-naïve women to undertake a preliminary Assessment Cycle [AC] [22]; secondly, utilizing the well described PIVET dosing-algorithms for ovarian stimulation [22-25], one of which is shown in Table 1; and thirdly, a commitment to single embryo transfer [SET] procedures for both fresh and frozen embryos across the entire age profile. Currently, in keeping with a widely encouraged practice across Australia and New Zealand, SET procedures at PIVET are currently at 91% of all cases [20]. In association with this, PIVET commits to a blastocyst culture system whenever 3 or more embryos are progressing on a Day-3 laboratory inspection, currently occurring in 90% of *in vitro* fertilization [IVF] cases. This means that there is also a high commitment to cryopreservation, which at PIVET is conducted using the Cryotop vitrification technique [26].

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, Puregon across the range 125-450 IU and Elonva in the narrow range of 200-400 IU. 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 her 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 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/m²		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 For Groups B and C with mid-range AFCs 9-19 follicles

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/m^2		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 For Groups D and E with low AFCs ≤8 follicles. The legend for all tables is under Table 1c.

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	Oocyte Donors & Oocyte Banking
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	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

2.2. Patient selection

Across the period January 2011 to December 2019, 3751 women entered into 10,728 treatments of various ART categories. Figure 1 is a flowchart showing the derivation of 2376 women who had an AC which also included an IGF profile. In the current study we are exploring the relevance of a range of poor-prognosis factors to the subsequent IVF treatment outcomes. From Figure 1 it can be seen that a total 3637 IVF treatments were initiated. These cycles utilized ICSI according to well reported PIVET protocols where indicated [27]. This included an IVF-ICSI Split model for unexplained infertility cases. The poor-prognosis criteria established from our former studies included advanced age of the woman ≥40 years; a low ovarian reserve (Table 1c; PIVET Algorithm Group D & E indicating an AFC ≤8 antral follicles and AMH level <10 pmol/L [4]; an IGF-1 level in the lowest quartile (<21 nmol/L [3,5]); incremental IVF treatment cycles (≥3 OPU cycles [28]); and poor-quality embryos resulting in zero blastocysts available or suitable for cryopreservation (nil frozen; Fz) [29]. These factors have been analyzed individually and in combination.

2.2.1. Patients utilizing rGH adjuvant therapy

Women categorized as likely to have a poor prognosis from previous IVF experience were informed and offered access to one of 3 adjuvants, namely DHEA, melatonin or rGH. Most women elected to take their chances without adjuvants, or selected the least expensive options, namely DHEA or melatonin. However, 646 of the 2376 women (27.2%) undertook a total of 1004 IVF cycles using rGH adjuvant therapy (27.6% of initiated cycles). DHEA was chosen by 790 women (33.3%) in 1189 treatment cycles (32.7%) (Figure 1). Both rGH and DHEA were used in combination by 529 women (22.3%) in 760 treatment cycles (20.9%). The use of melatonin was the subject of an earlier related report (Study 1) [15].

The rGH regimen utilized SciTropin (SciGen, Belrose, Australia) 0.3 mg self-injected subcutaneously daily beginning Day-3 of the pre-IVF cycle for ~45 days leading up to the human chorionic gonadotropin; hCG trigger, receiving rGH at precisely 1.0 IU per day prior to OPU. The information provided to the women includes the statement "approximately 35% of women do not achieve a live birth from current standard IVF regimens and many seek adjustments, adjuvants and add-ons attempting to improve their prognosis. There are 10 physiological areas of focus with more than 50 adjuncts currently described. However, none have proven benefit according to the highest statistical standards for evidence-based medicine; EBM [12,30]".

2.2.2. Patients utilizing Melatonin adjuvant therapy

Melatonin is an over-the counter agent, classified similarly to supplements of vitamins and minerals. It is a natural substance, derived in the pineal gland from the amino acid tryptophan under hypothalamic control for the regulation of circadian rhythms. For PIVET patients it was prescribed as a tablet of 3mg compounded by Wembley Pharmacy, Perth, Western Australia and was given in conjunction with Myo-inositol 1000 mg capsules, along with D-Chiro inositol 25mg/day for those women with polycystic ovary syndrome. The course of melatonin is given over the same 6-week period in conjunction with the rGH course. These supplements are powerful antioxidants which also have anti-inflammatory properties [31] with potential, albeit unproven benefits in IVF.

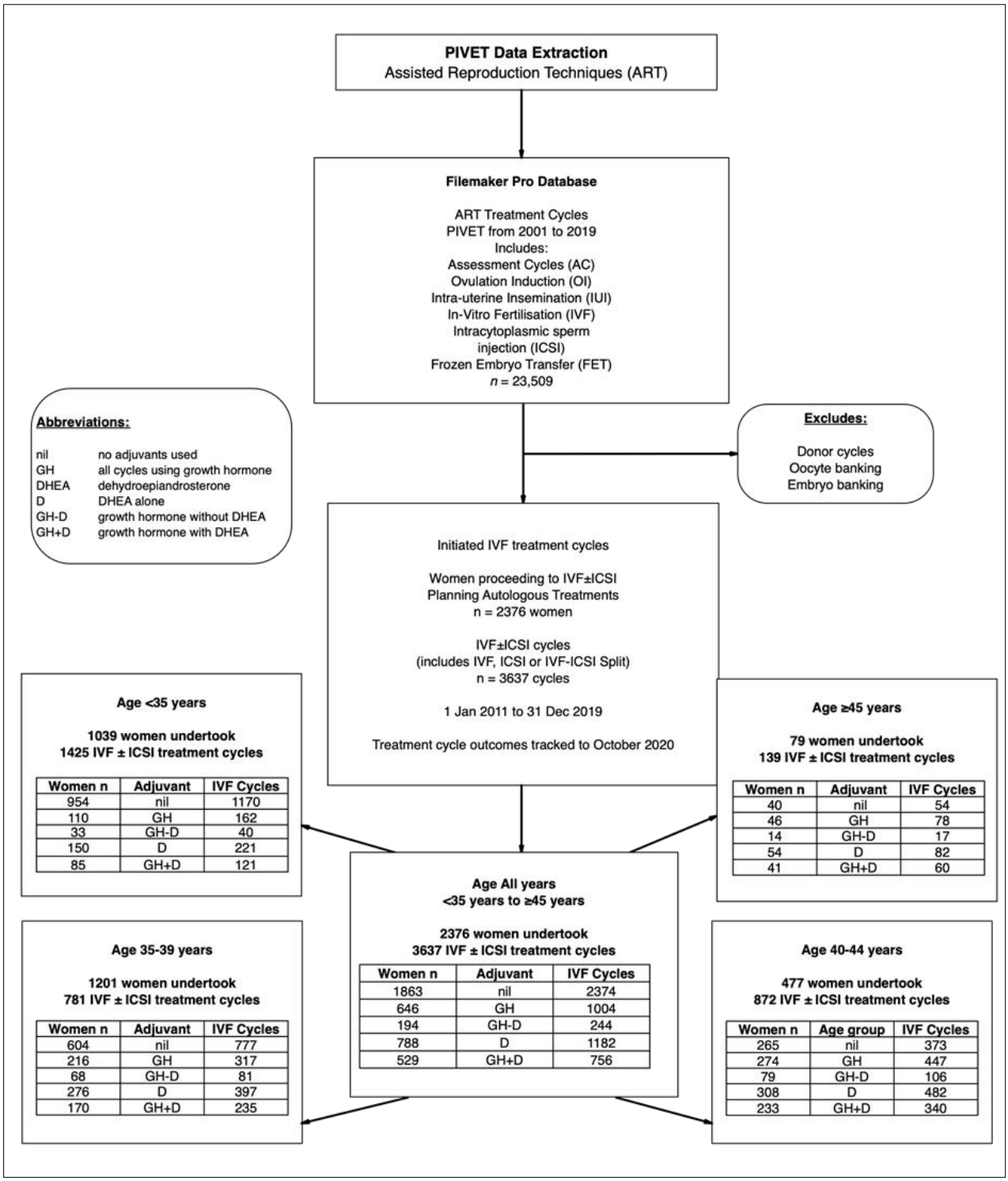

Figure 1 Flow diagram showing the derivation of 3637 IVF±ICSI autologous treatment cycles in 2376 women across the period 2011 to 2019. The cycles are distributed according to the woman's age at the initiation of treatment and whether she used the adjuvants growth hormone or dehydroepiandrosterone (DHEA). PIVET has undertaken IVF treatments since 1981 and the current Filemaker Pro Database was established in 2001

2.2.3. Patients utilizing DHEA adjuvant therapy

Dehydroepiandrosterone is a multifunctional adrenal prohormone which is compounded as a troche by Wembley Pharmacy according to an in-house PIVET recipe. Briefly, DHEA microparticles were dispersed in a sweetened and flavored mix of polyethylene glycol base (50-100 μM). This mix allows DHEA to release slowly and uniformly by the buccal sub-lingual route with proven androgenic responses [13]. The 50mg troches are cross scored enabling dosages

of 12.5 mg, 25mg, 37.5 mg and 50 mg according to androgen response profiles and side-effects such as hair growth and alopecia. The DHEA is utilized concurrently with the 6-week rGH regimen, but many patients will continue its use over 4-6 months to maintain normal androgen profiles.

2.3. IVF outcome parameters

2.3.1. Cycles initiated

Cycles initiated denotes those autologous IVF cycles which commence ovarian stimulation with gonadotrophins on Day-3. Approval requires that base-line parameters are met on day-2, namely serum gonadotrophins, being follicle stimulating hormone [FSH] and luteinizing hormone [LH] indicating the woman is not menopausal or in ovarian failure. In addition, her ovarian hormones must be at basal levels, namely estradiol [E2] level <150 pmol/L and Progesterone [P4] level <5 nmol/L along with her pituitary Prolactin <750 IU/L [22].

2.3.2. Cancelled cycles (Cancelln rate %)

Cancelled cycle indicates the IVF cycle is abandoned, mostly prior to the hCG trigger which is usually given around day-12. This usually arises from a lack of response to the ovarian stimulation with lack of E2 elevation and failure to detect ovarian follicles ≥16 mm by transvaginal pelvic ultrasound. Cycles with poor responses are maximally stimulated with FSH at 450 IU/day up to a maximum 16 days (day-19 of IVF cycle). Cycles may also be abandoned if there is evidence of premature ovulation (Rising LH, elevated P4 and marked fall in E2). This problem is unusual in current practice which routinely applies gonadotrophin releasing hormone [GnRH] antagonists [22]. Occasional causes of cancelled cycles may be patient-related issues such as intercurrent illness, domestic stresses and logistical problems related to travel from remote locations. Cases with zero oocytes recovered at OPU are also included in this category.

2.3.3. Oocytes retrieved

Oocytes retrieved denotes every oocyte cumulus complex [OCC] detected following ovarian follicle aspiration at ovum pick-up [OPU]. At PIVET this is undertaken by a single-lumen aspirating needle when follicle numbers >12 mm ≥5; and a double-lumen flushing needle when there are fewer follicles (32). Oocytes may be subsequently categorized as mature (metaphase II), immature (metaphase I) or germinal vesicle stage. Some OCCs will reveal zona-fragmentation, empty zona or oocyte degeneration. Only mature oocytes at the metaphase-II [MII] stage by the time of ICSI, being 4-6 hours post-OPU, are used for fertilization.

2.3.4. Oocyte utilization rate (O Utn %)

Oocyte utilization rate denotes the number of oocytes which fertilize and contribute to the formation of embryos which are utilized in a fresh embryo transfer [ET] procedure or a frozen embryo transfer [FET] procedure after a period of cryopreservation. This is factored for all oocytes, be they at MII, MI or the germinal vesicle stage, or even if they are subsequently shown to have degenerated or have fractured zona pellucidae. Specifically, this oocyte utilization rate is not the same as the oocyte fertilization rate which designates the number of embryos arising after oocytes are selected for insemination or ICSI; considered by us to be a less useful measure.

2.3.5. Increased monitoring

Increased monitoring relates to those women who have >12 oocytes recovered at OPU and are therefore classified as being at risk for ovarian hyperstimulation syndrome [OHSS]. Such women have daily contact with PIVET to discuss the woman's wellbeing, including measurement of her abdominal girth, description of urine characteristics (light or dark color and output), as well as blood test monitoring every 2nd or 3rd day of the luteal phase, with concern if E2 >6000 pmol/L and/or P4 >600 nmol/L. Such patients may return to PIVET for intravenous fluids and specific gravity monitoring of their urine. Occasional women will need paracentesis of ascites and hospitalization, although such events have reduced to 0.1% in recent years.

2.3.6. Embryo utilization rate (E Utn %)

Embryo utilization rate denotes the number of fertilized oocytes (defined at the two-pronuclear; 2PN stage), which are then utilized in a fresh ET procedure or subsequently in a FET procedure after a period of cryopreservation. PIVET protocols [22] means that ~85% of all oocytes retrieved undergo a corona-cumulus stripping process for ICSI, which enables identification of the maturational stage of the oocyte, particularly the MII oocyte which has released a single polar body. Those oocytes subjected to IVF-only, have the maturational stage presumed at the 18-hour PN-stage check when pipette-stripping occurs. If the 2-PN stage is identified, it is presumed that the oocyte must have been at the MII stage at OPU.

2.3.7. Embryos frozen (E Fz %)

Embryos frozen denotes the proportion of all embryos generated which are cryopreserved by vitrification. This is usually those embryos which reach the blastocyst stage on day-5 or day-6 with Gardner Grading at the level of 3BB or better, ideally 4AA or 5AA for best prognosis [29]. Sometimes all suitable embryos are cryopreserved in a "freeze-all" cycle to reduce the OHSS risk. The majority of IVF cycles in women classified as having a good prognosis can generate 3-4 high-grade embryos from ~10 oocytes recovered at OPU, meaning that 2 or 3 will be cryopreserved after the fresh SET procedure.

2.3.8. Freeze-all embryos

Freeze-all embryos denotes those cases where a fresh embryo transfer is not performed, mainly to reduce the risk of OHSS. Suitable embryos, mostly blastocysts, are committed to cryopreservation for future FETs. This option is considered when ≥15 oocytes are recovered at OPU or fresh ETs are deferred for other reasons (including when the woman is unwell, or embryo transfer is deferred awaiting procedures such as hysteroscopy or laparoscopy to correct pelvic conditions). Where embryo biopsy is undertaken for pre-implantation diagnosis a freeze-all process is undertaken on the biopsied blastocysts, awaiting the diagnostic report for a subsequent FET with normal embryos. The minimal grading considered suitable for cryopreservation is 3BB according to the Gardner & Schoolcraft grading system [29].

2.3.9. Pregnancy productivity rate (PP %)

Pregnancy productivity rate denotes the total number of pregnancies arising after both fresh ETs and FETs related to a single initiated cycle reaching the stage of OPU. This means the freeze-all or freeze-best embryo strategies do not prejudice the "pregnancy rate". It is for this reason we have developed such terminology rather than the oft-used cumulative pregnancy rate which, traditionally related to several OPU cycles [33]. Furthermore, the pregnancy productivity rate can be designated from the initiation of a treatment cycle (following the baseline blood test performed on cycle Day-2); from the stage of an OPU procedure where at least one oocyte is recovered; or from the stage of reaching an ET procedure (performed in either the fresh cycle or following cryopreservation). This parameter is different from the pregnancy productivity rate per each and every ET; considered by us to be a less useful measure, but nonetheless noted in the Tables of Outcomes.

2.3.10. Early pregnancy losses (EPLs); designated Miscarriage rate (Mis %)

Miscarriage rate denotes those pregnancies which do not advance to a livebirth and are invariably lost before 20-weeks "gestation", the division point for obstetric outcomes. At PIVET pregnancies are diagnosed provisionally at Day-19 of the luteal phase as "4-weeks" when serum ßhCG is detected >25 IU/l. At this stage the pregnancy is 'biochemical only" but is tracked each week until a transvaginal pelvic scan at 7-weeks denotes the presence of an intra-uterine gestational sac, expectantly with a definable viable fetus. However, at PIVET clinical pregnancy is diagnosed at week-5 if the ßHCG elevation is around 5 to 10-fold that of 4-weeks. The diagnosis of clinical pregnancy is strengthened if there is a further 2 to 5-fold rise along with associated appropriate levels of E2 and P4. PIVET has hormonal support strategies for those pregnancies with threatened miscarriage and suboptimal P4 levels [22]. In this context, miscarriage rates cover various early pregnancy losses and include pregnancies of unknown location (PUL), ectopic gestations, blighted ovum losses and terminations of abnormal or demised fetuses prior to gestational age 20-weeks. PULs may receive methotrexate at week-6 to week-7 if an intra-uterine fetus is not defined at trans-vaginal ultrasound and hormonal levels are suboptimal, hence a definite diagnosis is often not determined. In this study the miscarriage rate is given as all pregnancy losses (numerator) as a proportion of clinical pregnancies (denominator) defined at 5-weeks.

2.3.11. Live birth productivity rate (LBP %)

Live birth productivity rate (LBPR) denotes the total number of pregnancies arising after both fresh ETs and FETs related to a single initiated cycle reaching the stage of OPU and delivering after 20 weeks. As with pregnancy rates, this means freeze-all or freeze-best embryo strategies do not prejudice the "pregnancy rate". It is for this reason we have developed such terminology rather than the oft-used cumulative live birth rate which, traditionally related to several OPU cycles [33]. Each delivery is counted as one live birth, even where twins or higher-order multiples are delivered. Stillbirths are not included among the live births, being analyzed separately among perinatal losses. Fortunately, such adverse outcomes are now uncommon in Australia due to the SET policy [20]. Furthermore, PIVET records twinning rates at <2.0% with nil triplets or higher order multiples in recent years [21]. Furthermore, the live birth productivity rate can be designated from the initiation of a treatment cycle (following the baseline blood test performed on cycle Day-2); from the stage of an OPU procedure where at least one oocyte is recovered; or from the stage of reaching an ET procedure (performed in either the fresh cycle or following cryopreservation). This parameter is different from the live

birth productivity rate per each and every ET, considered by us to be a less useful measure. Nonetheless, the data is recorded in the tables of results.

2.4. Statistical evaluation

Data extractions from the Filemaker database were placed in Microsoft Excel spreadsheets and sorted according to the relevant tests. Thereafter the sorted data was placed in the application Past 4.03 (developed by Øyvind Hammer) [34] for statistical data analysis. This application also generated the Tables comprising the statistical summaries, finally placed in Microsoft Word for clearer display. The relationship among data means was examined by one-way ANOVA for overall comparison. The Kruskal-Wallis test was applied to examine equality between sample medians and Mann-Whitney applied for pairwise comparisons between individual sub-groups. Ratio comparisons between two 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. These many comparisons were conducted efficiently in the Past application. Following corrections, probability values of $p<0.01$ were considered significant for any test. As this data is retrospective by design, with wide variance and large kurtosis, hence several comparisons which were borderline, ranging 0.03 to 0.05; were classified as being of marginal significance. The Figures displayed for this study are derived from Excel v 16.42 (2020) and X-Diagram v 5.7 application (2021) developed by Vu Tien Thinh.

2.5. List of Abbreviations

The abbreviations shown in the methodology section of this report, mainly those used in the Tables and Figures, are listed here for clearer reference.

Adj	adjuvants
Cancn %	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
PP/In %	pregnancy productivity rate per initiated cycle
PP/O %	pregnancy productivity rate per oocyte pick-up (OPU)
PP/ET %	pregnancy productivity rate per embryo transfer procedure
Mis %	miscarriage rate (early pregnancy losses from week 5)
LBP/In %	live birth (L/B) productivity rate per initiated cycle (LBPR)
LBP/O %	live birth (L/B) productivity rate per oocyte pick-up (OPU)
LBP/ET %	live birth (L/B) productivity rate per embryo transfer procedure
LBPR	live birth productivity rate
rGH, G	recombinant growth hormone
DHEA, D	dehydroepiandrosterone
nGD	Neither rGH nor DHEA
AFC	antral follicle count (small follicles <10mm)
AMH	anti-Mullerian hormone
BMI	Body mass index kg/m^2
PIVET	registered acronym from programmed IVF & ET
n.s.	not significant
yrs	years

3. Results

3.1. Global outcomes

Table 2 Summary table covering the embryology and clinical outcomes of 3637 IVF±ICSI autologous treatment cycles initiated on 2376 women at PIVET across the years 2011-2019 and pregnancies tracked through 2020. The data is categorized according to use of the adjuvants growth hormone and/or DHEA and includes women of all ages, covering all grades of ovarian reserve depicted in Tables 1a, 1b and 1c.

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E						
Ages: all years	**Totals: all ages <35 years through to ≥45 years**					
Adjuvant	All	G	nGD	G-D	D	G+D
Cycles (n)	3637	1004	2374	244	1182	756
Women (n)	2376	646	1863	194	788	529
Cancellns %	0.3	0.5	0.2	0.0	0.5	0.7
O/OPU (n)	9.2	6.7	10.4	8.0	6.6	6.3
O Utn %	26.0	25.1	26.3	22.8	25.6	26.1
E Utn %	48.5	49.7	47.9	43.9	50.4	52.2
E Utn Stats	***adjuvants or combinations compared to nil adjuvants (n.s)***					
E Fz %	30.5	21.2	32.7	19.9	22.6	21.8
PP/ In %	46.5	22.9	57.5	26.2	24.3	22.0
PP/ OPU %	46.6	23.0	57.6	26.2	24.4	22.1
PP/ ET %	61.9	33.4	73.5	36.4	34.7	32.6
Mis %	23.7	29.1	21.8	25.0	29.6	30.7
LBP/ In %	35.4	16.2	44.9	19.7	17.1	15.2
LBP/ In Stats	***nil adjuvants better than G, D, or combinations (p<0.0001)***					
LP/ OPU %	35.6	16.3	45.0	19.7	17.2	15.3
LP/ ET %	47.2	23.7	57.4	27.3	24.5	22.6

The mean oocyte number retrieved from all the 3637 cycles was 9.2, ranging from 6.3 in the G+D adjuvant group to 10.4 in the group who received no adjuvants indicating no increase in oocytes at OPU from adjuvant use (Figure 2a).

The mean embryo utilization rate ranges narrowly between 43.9% to 52.2% and there are no significant differences among these rates according to the adjuvant used. However, the rate range is lower for younger women (<40 years) with mean rates ranging 46.3% to 49.5% (Table 2a) than for older women (≥40 years) with mean rates ranging 48.6% to 58.3%, p<0.001 (Table 2b). The live birth productivity rate ranges 15.2% to 19.7% across the adjuvant groups and these rates are significantly lower than the 44.9% rate for those women who had not used adjuvants (p<0.0001) (Figure 2b).

For the younger women, the mean oocyte numbers collected at OPU was 11.1 for women <35 years and lower at 9.4 for women aged between 35-39 years (Table 2a). For each age group the oocyte number was highest for women who did not receive adjuvants, hence it is apparent that adjuvants did not increase oocyte numbers among this younger group. Embryo utilization was 49.3% (ranging narrowly 48.5% to 50.9%) for women <35 years and 47.0% (ranging 36.5% to 49.9%) for the women aged 35-39 years. Notwithstanding the differences in rate ranges, at all ages <40 years the embryo utilization rate was not significantly increased using the adjuvants rGH or DHEA, neither alone nor in combination [Figure 2a]. Similarly, the mean live birth productivity rates per initiated cycle was 55.3% (ranging widely from 28.5% to 60.8%) for the younger women <35 years and 34.5% (ranging 19.8% to 40.0%) for the older group. In

each case the highest rates were recorded for those women who received no adjuvants indicating no benefit from the adjuvants in women <40 years [Figure 2b].

Table 2a Embryology and clinical outcomes from the younger groups of women (comprising 1039 with ages <40 years) who had 1425 IVF±ICSI treatment cycles during 2011 to 2019 embracing all ovarian reserve categories depicted in Tables 1a, 1b and 1c.

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E												
Ages <40 yrs	**<35 years**						**35-39 years**					
Adjuvant	All	G	nGD	G-D	D	G+D	All	G	nGD	G-D	D	G+D
Cycles (n)	1425	162	1170	40	221	121	1201	317	777	81	397	235
Women (n)	1039	110	954	33	150	85	781	216	604	68	276	170
Cancellns %	0.1	0.0	0.2	0.0	0.0	0.0	0.4	0.3	0.3	0.0	0.5	0.4
O/OPU (n)	11.1	8.8	11.6	11.3	8.3	8.0	9.4	7.5	10.4	9.0	7.4	7.0
O Utn %	26.9	23.9	27.5	24.1	23.6	24.0	25.3	23.9	25.2	19.5	25.8	25.8
E Utn %	49.3	48.5	49.5	44.7	47.7	50.9	47.0	45.6	46.4	36.5	48.2	49.9
E Ut^n Stats	***adjuvants or combinations vs nil adjuvants (n.s.)***											
E Fz %	35.7	30.2	36.5	31.1	27.8	30.2	29.6	20.2	31.3	14.7	24.4	22.9
PP/ In %	69.4	44.4	75.6	70.0	38.0	36.4	45.3	30.9	50.7	29.6	34.3	31.5
PP/ OPU %	69.5	44.4	75.8	70.0	38.0	36.4	45.5	31.0	50.8	29.6	34.4	31.6
PP/ ET %	88.1	67.9	93.8	96.6	53.2	57.9	60.1	42.6	65.3	38.7	47.4	44.3
Mis %	20.4	23.6	19.7	14.3	25.0	29.5	23.9	29.6	21.1	33.3	28.7	28.4
LBP/ In %	55.2	34.0	60.8	60.0	28.5	25.6	34.5	21.8	40.0	19.8	24.4	22.6
LBP/ In Stats	***adjuvants or combinations vs nil adjuvants (n.s.)***											
LP/ OPU %	55.3	34.0	60.9	60.0	28.5	25.6	34.6	21.8	40.1	19.8	24.6	22.6
LP/ET %	70.1	51.9	75.3	82.8	39.9	40.8	45.7	30.0	51.6	25.8	33.8	31.7

For the older women, the mean oocyte numbers collected at OPU was 6.5 for women aged 40-44 years and lower 4.1 for women aged between ≥45 years (Table 2b). These numbers were significantly lower than the oocyte numbers in the younger group (<40 years; $p<0.0001$) despite the older women receiving higher FSH dosing according to the PIVET Algorithm (to a maximum 450 IU daily). However, for each age group it is apparent that adjuvants did not increase oocyte numbers among either category of this older group. The embryo utilization rates were a little higher for the older women (48.6% to 57.0%) compared with the younger (47.0% to 49.3%, $p<0.001$) but the rates were not significantly different among the adjuvant categories for each delineated age group excepting for those women in the age range 40-44 years. That group had an embryo utilization rate of 48.6% ranging 50.9% to 54.4% for those women using adjuvants compared with 43.7% for those who did not, being a highly significant difference favoring both the rGH and DHEA adjuvants ($p<0.0001$) [Figure 2b]. However, for women aged ≥45 years the mean embryo utilization rate was 57.0% and showed no significant variation among the adjuvant groups, including 56.1% for no adjuvants [Figure 2b]. Similarly, the mean live birth productivity rates per initiated cycle in the age group 40-44 years was 10.0%, ranging 7.5% to 8.8% for those women using adjuvants, and 11.8% for those who used no adjuvants. Among the oldest women aged ≥45 years, live births rates were very low at nil to 1.7%, with the only births occurring in those women who used either rGH, or DHEA or the combination [Table 2b, Figure 3b].

The LBPRs were significantly higher for the younger women (55.2% down to 34.5%) compared to the older women (10.0% down to 0.7%, $p<0.0001$) and the adjuvant categories showed no significant benefit over nil adjuvants at each age category; in fact; nil adjuvants proved significantly better than any of the adjuvants alone or in combination, particularly for younger women (Figure 3b, $p<0.005$ for women <40 years and $p<0.0001$ overall).

Table 2b Embryology and clinical outcomes from the older groups of women comprising 477 who had 872 IVF±ICSI treatment cycles during 2011 to 2019 embracing all ovarian reserve categories depicted in Tables 1a, 1b and 1c.

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E												
Ages ≥40 yrs	**40-44 years**						**≥45 years**					
Adjuvant	All	G	nGD	G-D	D	G+D	All	G	nGD	G-D	D	G+D
Cycles (n)	872	447	373	106	482	340	139	78	54	17	82	60
Women (n)	477	274	265	79	308	233	79	46	40	14	54	41
Cancellns %	0.6	0.9	0.0	0.0	0.8	1.2	0.0	0.0	0.0	0.0	0.0	0.0
O/OPU (n)	6.5	5.8	7.5	6.5	5.7	5.6	4.1	4.1	4.5	4.9	3.8	3.7
O Utn %	24.9	26.5	23.6	25.7	26.2	26.8	29.2	28.5	28.3	21.4	30.2	30.8
E Utn %	48.6	53.5	43.7	50.9	53.9	54.4	57.0	55.6	56.1	52.9	57.0	55.6
E Utⁿ Stats	***higher rates with G±D adjuvants vs nil adjuvants (p<0.0001)***											
E Fz %	18.5	18.1	18.6	19.1	18.2	17.7	16.4	13.6	21.1	8.8	12.7	14.5
PP/ In %	17.8	13.2	22.5	11.3	13.7	13.8	1.4	1.3	1.9	0.0	1.2	1.7
PP/ OPU %	17.9	13.3	22.5	11.3	13.8	14.0	1.4	1.3	1.9	0.0	1.2	1.7
PP/ ET %	25.0	19.3	30.2	15.8	19.9	20.4	2.4	2.2	3.2	0.0	2.0	2.8
Mis %	43.9	35.6	47.6	33.3	37.9	36.2	50.0	0.0	100.0	0.0	0.0	0.0
LBP/ In %	10.0	8.5	11.8	7.5	8.5	8.8	0.7	1.3	0.0	0.0	1.2	1.7
LBP/ In Stats	***adjuvants or combinations vs nil adjuvants (n.s.)***											
LP/ OPU %	10.0	8.6	11.8	7.5	8.6	8.9	0.7	1.3	0.0	0.0	1.2	1.7
LP/ET %	14.0	12.4	15.8	10.5	12.4	13.0	1.2	2.2	0.0	0.0	2.0	2.8

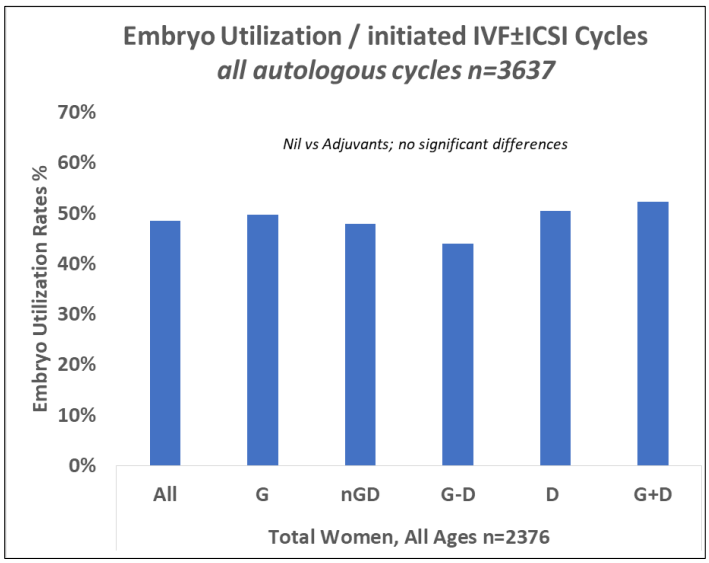

Figure 2a Bar chart depicting embryo utilization rates from the global group of 3637 IVF±ICSI treatment cycles undertaken on 2376 women during 2011 to 2019 embracing all age groups and ovarian reserve categories. A total 33213 autologous oocytes were collected, of which 24432 were inseminated or injected, generating a total 17815 embryos. Of these embryos, Fresh ETs utilized 3218 embryos and frozen ETs utilized 2236 embryos. No significant differences were shown between nil vs rGH or DHEA adjuvants, nor combinations

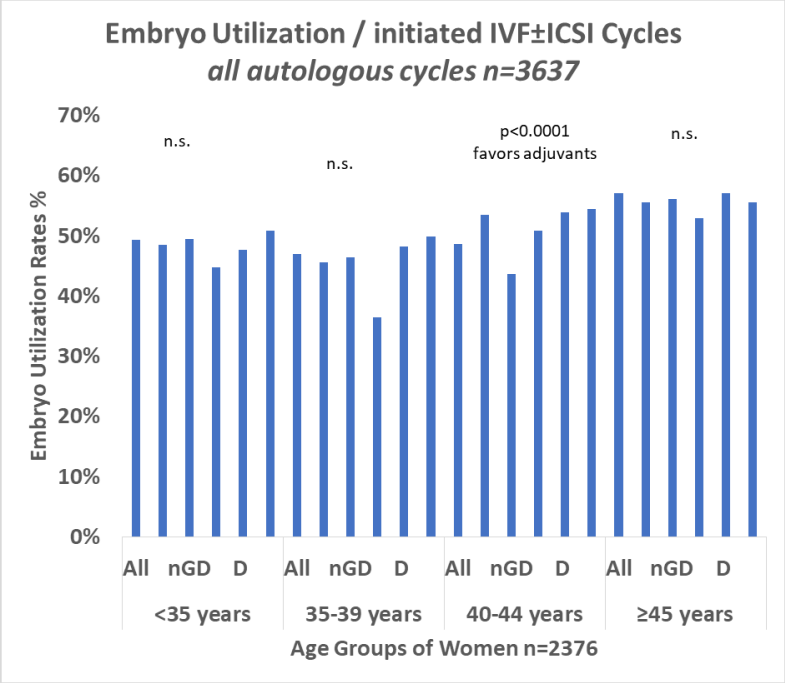

Figure 2b Bar chart depicting embryo utilization rates categorized according to age groups. A significant improvement in embryo utilization was detected in the age group of 40-44 years alone, from rGH ± DHEA ($p<0.0001$). Younger and older age groups showed no significant benefit from these adjuvants

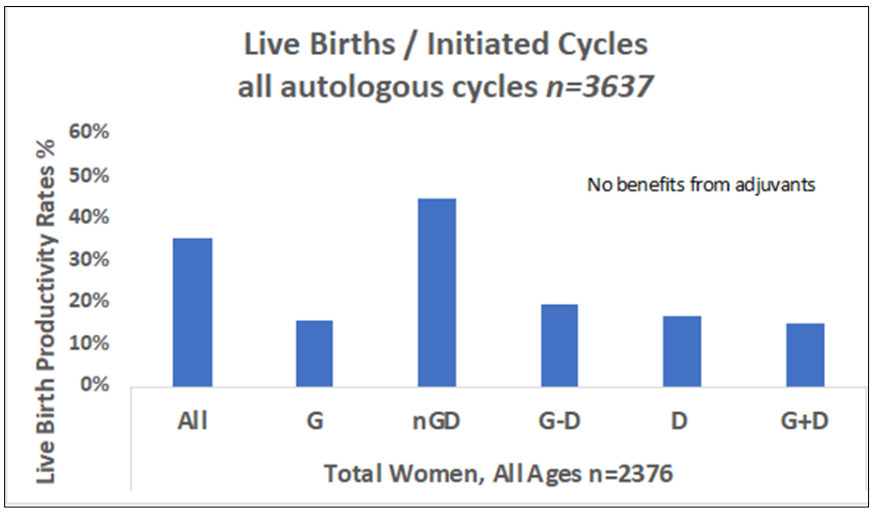

Figure 3a Bar chart depicting live birth productivity rates from the global group of 3637 IVF±ICSI treatment cycles initiated on 2376 women during 2011 to 2019 embracing all age groups and ovarian reserve categories. Overall, there were 769 pregnancies from 3218 fresh ETs and 921 from 2318 frozen ETs giving a pregnancy productivity rate per initiated cycle of 46.5%. Following early pregnancy losses, the comparable live birth rates were 598 live births from fresh ETs (18.6%) and 703 live births from frozen ETs (30.3%) generating an overall live birth productivity rate of 35.4% per initiated cycle. Neither rGH nor DHEA nor combinations showed any benefit over nil adjuvants

Figure 3a shows the live birth rates from the global dataset for all the autologous IVF±ICSI treatment cycles with respect to the use of the adjuvants rGH or DHEA or the rGH/DHEA combination. The treatment outcomes show that there were no discernible benefits from the use of adjuvants. In fact, the treatment cycles with nil adjuvants had live birth outcomes significantly better than the adjuvant groups ($p<0.0001$). Among the age groups, those women aged under 40 years had significantly better outcomes without adjuvants ($p<0.001$ for <35 years and $p<0.005$ for those between ages 35-39 years) [Table 2a]. There were no significant differences demonstrated with or without adjuvants for those women ≥40 years [Table 2b]. In summary, it can be seen from Figure 3b that neither rGH, nor DHEA or combined rGH/DHEA had any improvement over nil adjuvants. In keeping with world-wide reports, the rate of live births among women ≥45

years is very low (<2%) for those utilizing their own autologous, age-matched oocytes (ref) and our study failed to show any significant improvement from the adjuvants; albeit each of the 3 live births recorded had utilized rGH or DHEA, or both adjuvants.

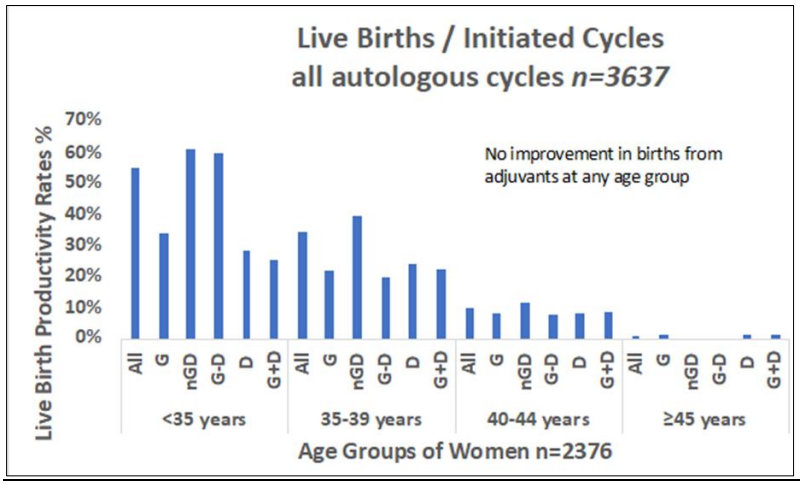

Figure 3b Bar chart depicting live birth productivity rates categorized according to age groups. No significant benefits could be shown from the use of adjuvants rGH, nor DHEA nor combinations among any of the age groupings. Live birth rates were very low among women aged ≥45 years; of interest each one of the 3 live births had utilised rGH alone or DHEA alone or rGH and DHEA together, respectively

3.2. Clinical prognostic criteria

The most relevant outcomes measured in this study of IVF±ICSI treatments include oocyte numbers, fertilization rates, embryo utilization rates, pregnancy rates and livebirth outcomes. The main criteria impacting on the two key outcomes of embryo utilization rates and live birth outcomes include the woman's age, her ovarian reserve, and the quality of the resulting embryos. The age factor has been analyzed in the Tables and Figures within Section 2, and other criteria impacting on prognosis are presented in this Section 3.2, albeit the Tables and Figures are not presented, being available at request from the corresponding author.

3.2.1. Ovarian reserve parameters

Ovarian reserve can be classified into specific groupings according to the AFC numbers shown in the PIVET FSH-dosing algorithm depicted in Table 1. These groups range from the highest A group with AFC numbers >20 antral follicles (with sub-classifications A, A+ and A++) shown in Table 1a, progressively downwards to the lowest Group E with <5 antral follicles shown in Table 1c. For this study a high ovarian reserve embraces all Groups A to C (embracing Groups A++, A+, A, B and C, meaning ≥ 9 antral follicles shown in Tables 1a and 1b). A low ovarian reserve embraces Groups D and E, meaning <9 antral follicles shown in Table 1c).

Total AFC Groups A to E

Table 2, which summarized the outcomes of 2376 women who undertook 3637 IVF±ICSI treatment cycles, included women from all the AFC Groups A (with ≥20 antral follicles and/or AMH levels >20 pmol/l, Table 1a) through to E (with ≤4 antral follicles and/or AMH <5 pmol/l, Table 1c). An average of 9.2 oocytes were recovered at OPU overall and mean numbers ranged from 6.3 to 8.0 across the adjuvant groupings with no significant differences demonstrated and no improvement over nil adjuvants with mean number 10.4 oocytes. Overall, 26.0% of oocytes contributed to the formation of usable embryos designated as the embryo utilization rate which was 48.5%. As noted, there were no significant differences in embryo utilization across the various adjuvant groupings. The LBPR per initiated treatment cycle showed an average of 35.4% across all ages and adjuvant groupings but neither rGH, DHEA nor combinations showed any benefit over the nil adjuvant group. In fact, as shown in Figure 3a, the nil adjuvant group displayed the highest live birth rate (LBPR/Initiated cycle 42.0%) being significantly higher than each of the adjuvant groupings ($p<0.0001$).

High Ovarian Reserve; represented by high AFC and AMH

Categorizing the women with high AFC (and AMH) groups A to C shown in Tables 1a and 1b, indicates 1907 women undertook 2707 IVF±ICSI cycles showing that an average 10.4 oocytes at OPU were recovered overall. The mean levels

ranged 8.9 to 11.6 across the adjuvant categories without any statistical significance. The resultant embryo utilization rate overall was 47.1% and live birth productivity rate was 46.1%. Adjuvants did not improve embryo utilization overall across the age groups, but both rGH and DHEA showed higher rates in the group aged 40-44 years (rGH 44.4%, DHEA 45.5% rGH+DHEA 45.7% vs Nil 37.8%, p<0.001). Nonetheless, live birth productivity rates were significantly highest for nil adjuvants rather than either rGH or DHEA (49.2% vs 22.7% and 24.9% respectively, *p<0.0001*). Of interest there were no live births from the 42 treatment cycles undertaken for women age ≥45 years, despite an embryo utilization rate of 44.4% which was similar with or without adjuvants.

Low Ovarian Reserve; represented by low AFC and AMH

Categorizing the women with low AFC (& AMH) groups D & E shown in Table 1c, indicated 708 women undertook 1260 IVF±ICSI cycles showing that an average 5.6 oocytes at OPU were recovered overall. The mean levels ranged 4.3 to 6.8 across the adjuvant categories without any statistical significance. The resultant embryo utilization rate overall was 54.1% and live birth productivity was 20.6%. Growth hormone alone improved embryo utilization overall (from 54.1% nil adjuvants to 60.0%, p<0.005), most prominently in the 40-44 years group. However, live birth productivity rates were significantly highest for nil adjuvants rather than rGH or DHEA (31.7% vs 10.7% and 10.6% respectively; p<0.0001). For women ≥45 years despite embryo utilization of 54.1 %, the live birth productivity per initiated cycle was 1.0% overall, occurring in women given either rGH or DHEA and the highest live birth productivity rate per embryo transfer was 5.0% in this oldest group given both rGH and DHEA adjuvants combined.

3.2.2. Low IGF profile; represented by IGF-1 levels

From the category of low IGF-1 levels (<21 nmol/L), 1050 women undertook 1780 IVF±ICSI cycles showing that an average 9.8 oocytes / OPU were recovered overall. The mean levels ranged 6.7 to 9.8 oocytes across the adjuvant categories with the highest number from nil adjuvants. The resultant embryo utilization rate was 48.9% and live birth productivity rate was 37.4% overall. There were no significant differences between nil adjuvants vs rGH or DHEA, neither the combination of adjuvants. Furthermore, live birth productivity rates were significantly highest for nil adjuvants rather than rGH or DHEA (37.4% vs 15.3% and 20.2% respectively; p<0.0001). This effect was most pronounced for the younger women, those <40 years. In summary, adjuvants showed no benefit for women with a low IGF profile.

3.2.3. Recurrent Implantation Failure

From the category of Women with ≥3 OPU's without a pregnancy success, designated RIF, 556 women undertook 982 IVF±ICSI cycles showing that an average 7.6 oocytes / OPU were recovered overall. The mean levels ranged 6.5 to 8.5 oocytes across the adjuvant categories with the highest number from nil adjuvants. The resultant embryo utilization rate was 47.9% and live birth productivity rate was 17.5% overall. There were no significant differences in embryo utilization between nil vs rGH or DHEA, neither with the combination. Additionally, there were no significant improvements in LBPR with the adjuvants rGH or DHEA vs nil adjuvants; in fact, the highest LBPRs were seen without adjuvants, especially for the youngest women, <35 years (p<0.05).

3.2.4. Oocyte numbers at OPU

The PIVET algorithm was designed primarily to reduce the risk of ovarian hyperstimulation syndrome for women categorized as "good responders" by limiting responses to <15 follicles. In fact, the majority of women were shown to generate 8-12 follicles and retrieve that number of oocytes. The algorithm also had a secondary design to apply higher FSH dosages to optimize oocyte numbers for women categorized as "poor responders". The relevance of oocyte numbers retrieved at OPU was therefore considered as a potential factor for embryological and clinical outcomes. For this analysis oocyte number were categorized as high (meaning ≥5 oocytes were recovered) or low (meaning <5 oocytes were recovered.

High oocyte numbers: ≥5 oocytes recovered

In the category of women retrieving ≥5 oocytes at OPU, 1970 women undertook 2723 IVF±ICSI cycles showing that an average 11.4 oocytes / OPU were recovered overall. The mean levels ranged 9.4 to 11.4 oocytes across the adjuvant categories with the highest number from nil adjuvants. The resultant embryo utilization rate was 46.9% and live birth productivity rate was 43.7% overall. There were no significant differences in embryo utilization between Nil vs rGH or DHEA, nor the combination. Adjuvants did not improve the live birth productivity rates, on the contrary nil adjuvants had significantly higher rates than either rGH or DHEA (43.7% vs 23.7% and 24.9% respectively, p<0.0001).

Low numbers: <5 oocytes recovered

In the category of women retrieving <5 oocytes at OPU, 597 women undertook 886 IVF±ICSI cycles showing that an average of only 2.4 oocytes / OPU were recovered overall. The mean levels ranged 2.1 to 2.4 oocytes across the adjuvant categories with the highest number from nil adjuvants. The resultant embryo utilization rate was 72.8% and live birth productivity rate/ initiated cycle 10.9% overall. There was a significantly higher embryo utilization in favor of rGH 78.0% vs nil adjuvants 72.1% ($p<0.02$) in the total group, but not at the individual age groupings (n.s.). Adjuvants did not improve the live birth productivity rates/ initiated cycle; on the contrary nil adjuvants had significantly higher rates than either rGH or DHEA in the total group (14.6% vs 5.9% and 6.1% respectively, $p<0.005$). Among the delineated age groups, nil adjuvants showed significantly higher livebirth productivity in the 35-39 years age group than either rGH or DHEA (24.5% vs 9.3% and 6.2% respectively; $p<0.01$).

3.3. Poor Embryo quality

Most studies reporting on the prognosis for IVF have applied measures from the Bologna Criteria focusing on the Poor Ovarian Responder [POR)]. However, the POR criteria include the age of the woman and the number of oocytes arising from maximal ovarian stimulation without consideration of oocyte quality. In this study we have considered this question by analyzing embryo utilization and ensuing livebirth rates for those women whose embryos do not develop sufficiently well to enable the consideration of any for cryopreservation (nil Fz). In this category of women whose embryo quality gradings were too poor to enable any embryos to undergo cryopreservation, the live birth rates are derived solely from fresh embryo transfers. Notwithstanding that no FETs were carried out among these cases, the data is still placed in the livebirth productivity category, with the understanding that this relates to fresh transfers + nil fz.

3.3.1. Overall outcomes (embracing all AFC categories A to E)

Table 3 Summary table as a sub-category covering the embryology and clinical outcomes of 1727 IVF±ICSI autologous treatment cycles initiated on 1135 women who demonstrated poor embryo quality. This global group includes women across all ovarian reserve categories who, after fresh single embryo transfer, had no residual embryos considered suitable for cryopreservation

Autologous IVF±ICSI Cycles during 2011-2019: all AFC Groups A to E; nil embryos Fz						
Ages: all years	**Totals: all ages <35 years through to ≥45 years**					
Adjuvant	All	G	nGD	G-D	D	G+D
Cycles (n)	1727	668	928	154	755	511
Women (n)	1135	457	758	132	524	372
Cancell^{n}s %	0.7%	0.7%	0.4%	0.0%	0.8%	1.0%
O/OPU (n)	6.3	5.1	7.2	5.9	4.9	4.8
O Utn %	14.7%	20.3%	12.1%	18.7%	20.0%	20.9%
E Utn %	39.3%	47.3%	33.8%	44.5%	47.5%	48.5%
E Ut^{n} Stats	**adjuvants G, D, G+D better than nil adjuvants ($p<0.0001$)**					
E Fz %	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
PP/ In %	13.5%	10.8%	16.6%	12.3%	9.9%	10.4%
PP/ OPU %	13.6%	10.9%	16.7%	12.3%	10.0%	10.5%
PP/ ET %	19.1%	15.3%	23.0%	16.8%	14.0%	14.9%
Mis %	27.4%	29.2%	21.4%	15.8%	36.0%	34.0%
LBP/ In %	9.8%	7.6%	13.0%	10.4%	6.4%	6.8%
LBP/ In Stats	***nil adjuvants better than G, D, or combinations ($p<0.0001$)***					
LP/ OPU %	9.9%	7.7%	13.1%	10.4%	6.4%	6.9%
LP/ ET %	13.8%	10.9%	18.1%	14.2%	8.9%	9.9%

The overall outcomes for autologous treatment cycles across all age groups whose treatment cycles generated zero embryos for cryopreservation are summarized in Table 3.

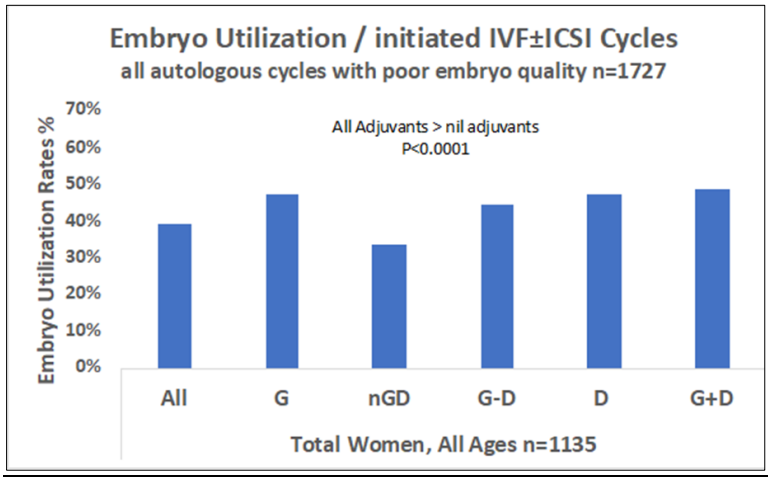

Figure 4a Bar chart depicting embryo utilization rates from the global group of 1727 IVF±ICSI treatment cycles undertaken on 1135 women where embryo quality was deemed poor such that no embryos were cryopreserved. The study period was during 2011 to 2019 and embraced all age groups and ovarian reserve categories. A total 10760 autologous oocytes were collected, of which 6488 were inseminated or injected, generating a total 3902 embryos. Of these embryos, Fresh ETs utilized 1596 embryos and there were nil embryos available for frozen ETs. No significant differences were shown between nil vs rGH or DHEA adjuvants nor combinations. All adjuvants RGH, DHEA and combined rGH+DHEA improved embryo utilization rates p<0.0001

3.3.2. Age influence with poor embryo quality (nil Fz)

The overall influence of age among the women deemed to have poor embryo quality is shown in Figure 4b for embryo utilization and Figure 5b for live birth rates.

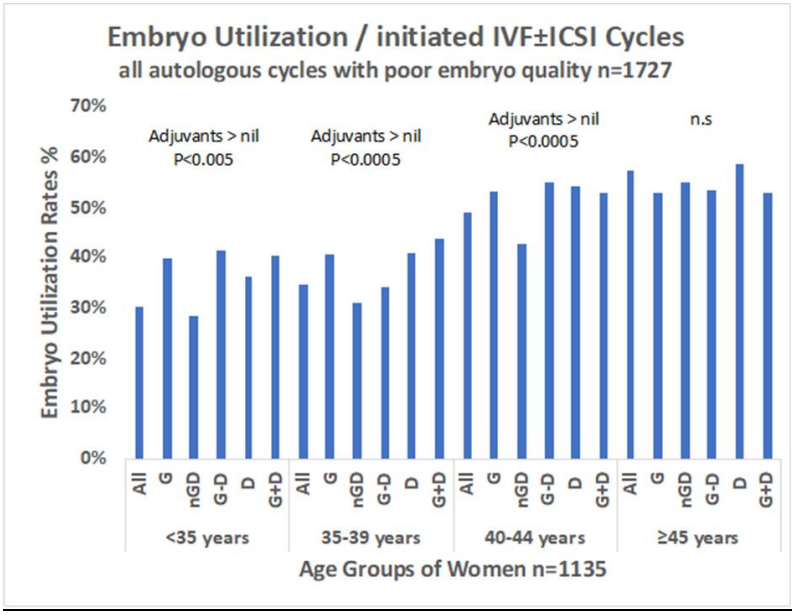

Figure 4b Bar chart depicting embryo utilization rates categorized according to age groups among women with poor embryo quality. Such women had no embryos cryopreserved and only fresh ETs were undertaken. Both rGH and DHEA adjuvants, alone or in combination had higher embryo utilization rates at each of the age groupings excepting for those women aged ≥45 years

These indicate 1135 women, being 47.8% of the study group, undertook 1727 IVF±ICSI cycles showing that an average of 6.3 oocytes / OPU were recovered overall. The mean levels ranged 5.1 to 7.2 oocytes across the adjuvant categories with the highest number from nil adjuvants. Overall, the embryo utilization rate averaged 39.3% with mean embryo utilization rates ranging 33.8% for nil adjuvants to 48.5% for combined rGH with DHEA adjuvants, a rate which is significantly increased ($p<0.0001$) [Figure 4a]. In keeping with the definition for poor embryo quality, it can be seen that nil embryos were cryopreserved (E Fz 0.0%). However, notwithstanding the improved embryo utilization rate with rGH adjuvant, the live birth rate, averaging 9.8%, was not improved by either of the adjuvants, neither alone nor in combination. The LBPR ranged 6.4% to 13.0%, the highest rate being for those women with nil adjuvants [Figure 5a].

Younger women

The embryology and clinical outcomes from those women aged under 40 years is shown in Table 3a, subcategorized to two groups – those under 35 years and those aged 35-39 years.

Table 3a The data from Table 3 is presented for the 724 younger women <40 years who initiated 1025 IVF±ICSI autologous treatment cycles who were deemed to have poor embryo quality with nil having any frozen embryo transfers. The women are sub-classified according to the youngest group <35 years and the older group ranging 35-39 years

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E; nil embryos Fz												
Ages <40 yrs	**<35 years**						**35-39 years**					
Adjuvant	All	G	nGD	G-D	D	G+D	All	G	nGD	G-D	D	G+D
Cycles (n)	476	80	350	15	111	64	549	201	303	50	230	150
Women (n)	352	57	297	13	78	45	372	146	250	45	167	114
Cancell^{ns} %	0.4	0.0	0.6	0.0	0.0	0.0	0.9	0.5	0.7	0.0	0.9	0.7
O/OPU (n)	7.8	5.4	8.3	6.8	5.6	4.9	6.8	5.9	7.7	7.3	5.5	5.5
O Ut^{n} %	9.6	14.8	9.1	14.7	13.6	15.0	13.3	18.0	11.3	15.9	17.7	18.8
E Ut^{n} %	30.5	40.3	28.8	41.7	36.3	40.5	35.0	40.8	31.0	34.3	41.1	44.0
E Ut^{n} Stats	***adjuvants G, D G+D vs nil adjuvants (p<0.005)***											
E Fz %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PP/ In %	19.5	20.0	22.0	46.7	13.5	14.1	14.8	14.9	16.5	18.0	13.0	14.0
PP/ OPU %	19.6	20.0	22.1	46.7	13.5	14.1	14.9	15.0	16.6	18.0	13.2	14.1
PP/ ET %	28.4	31.4	30.9	58.3	20.3	23.7	20.6	20.1	22.7	23.1	18.1	19.3
Mis %	19.4	12.5	16.9	0.0	26.7	22.2	19.8	30.0	10.0	22.2	33.3	33.3
LBP/ In %	15.8	17.5	18.3	46.7	9.9	10.9	11.8	10.4	14.9	14.0	8.7	9.3
LBP/ In Stats	***adjuvants or combinations vs nil adjuvants (n.s.)***											
LP/ OPU %	15.8	17.5	18.4	46.7	9.9	10.9	11.9	10.5	15.0	14.0	8.8	9.4
LP/ET %	22.9	27.5	25.7	58.3	14.9	18.4	16.5	14.1	20.5	17.9	12.0	12.8

The age distribution is categorized as younger women (<40 years) subclassified into 2 groups as <35 years and 35-39 years, and older women subclassified into 2 groups as 40-44 years and ≥45 years.

These indicate 724 women undertook 1025 IVF±ICSI cycles showing that an average of 7.8 oocytes / OPU were recovered from women <35 years and 6.8 oocytes from women aged 35-39 years. The mean levels ranged 5.4 to 8.1 oocytes across the adjuvant categories with the highest number from nil adjuvants. Overall, the embryo utilization rate averaged 35.0%. Compared to nil adjuvants rates were higher for RGH, particularly for the youngest women, in the <35 years age group (40.3% vs 28.8%; $p<0.01$) and highly significant for the combined <40 years group ($p<0.0001$). DHEA also showed significant benefit for the combined <40 years group [$p<0.0001$). Live birth productivity rates are shown

in Table 9a along with Figure 9b. Notwithstanding the rGH and DHEA benefits on embryo utilization, no beneficial effect was demonstrated on live birth rates which were 15.8% for women <35 years and 11.8% for those aged 35-39 years. There were no significant differences overall among the various LBPRs for those who did, or did not, receive adjuvants, albeit that the youngest women using rGH alone (without added DHEA) showed the highest rate of 46.7%, a rate derived from 7 livebirths from 15 treatment cycles ($p<0.05$).

Older women

The outcomes for older women (aged ≥40 years) who had zero embryos Fz are depicted in Table 3b with Embryo utilization rates shown in Figure 4a.

Table 3b The data from Table 3 is presented for the 411 older women ≥40 years who initiated 702 IVF±ICSI autologous treatment cycles who were deemed to have poor embryo quality with nil having any frozen embryo transfers. The women are sub-classified according to the younger group 40-44 years and the oldest group aged ≥45 years

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E; nil embryos Fz												
Ages ≥40 yrs	**40-44 years***						**≥45 years**					
Adjuvant	All	G	nGD	G-D	D	G+D	All	G	nGD	G-D	D	G+D
Cycles (n)	585	319	233	75	341	243	117	68	42	14	73	54
Women (n)	339	210	176	60	230	176	72	44	35	14	49	37
Cancellns %	0.9	1.3	0.0	0.0	1.2	1.6	0.0	0.0	0.0	0.0	0.0	0.0
O/OPU (n)	5.1	4.7	5.6	5.0	4.7	4.7	3.5	3.6	3.8	4.8	3.2	3.3
O Utn %	21.3	23.0	19.0	22.1	23.3	23.3	26.0	24.6	23.8	20.9	28.0	26.0
E Utn %	49.3	53.4	43.1	55.0	54.6	52.9	57.7	53.1	55.1	53.8	58.9	52.9
E Utⁿ Stats	***higher rates with G, D, G+D adjuvants vs nil adjuvants (p<0.0005)***											
E Fz %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PP/ In %	9.9	7.8	11.2	4.0	8.5	9.1	1.7	1.5	2.4	0.0	1.4	1.9
PP/ OPU %	10.0	7.9	11.2	4.0	8.6	9.2	1.7	1.5	2.4	0.0	1.4	1.9
PP/ ET %	13.4	10.9	15.1	5.6	11.5	12.5	2.7	2.5	3.6	0.0	2.2	3.1
Mis %	50.0	40.0	53.8	33.3	44.8	40.9	50.0	0.0	100.0	0.0	0.0	0.0
LBP/ In %	5.0	4.7	5.2	2.7	4.7	5.3	0.9	1.5	0.0	0.0	1.4	1.9
LBP/ In Stats	***adjuvants or combinations vs nil adjuvants (n.s.)***											
LP/ OPU %	5.0	4.8	5.2	2.7	4.7	5.4	0.9	1.5	0.0	0.0	1.4	1.9
LP/ET %	6.7	6.5	7.0	3.7	6.3	7.4	1.4	2.5	0.0	0.0	2.2	3.1

These indicate 411 women undertook 702 IVF±ICSI cycles showing that an average of 5.1 oocytes / OPU were recovered from women 40-44 years and 3.5 oocytes from women aged ≥45 years. The mean levels ranged 3.3 to 5.3 oocytes across the adjuvant categories with the highest number from nil adjuvants. Overall, the embryo utilization rate averaged 49.3% for the younger and 57.7% for the oldest women. Compared to nil adjuvants rates were significantly higher for both rGH and DHEA as well as combined rGH+DHEA for both of the higher age groupings ($p<0.0005$). Live birth rates were very low among these women of advanced age being 5.0% for the younger and 0.9% for the older group of women (Figure 9b). Furthermore, there were no significant benefits from either of the adjuvants, alone or in combination (rGH 4.7%, DHEA 4.7% and nil adjuvants 5.2% for the younger group, n.s.).

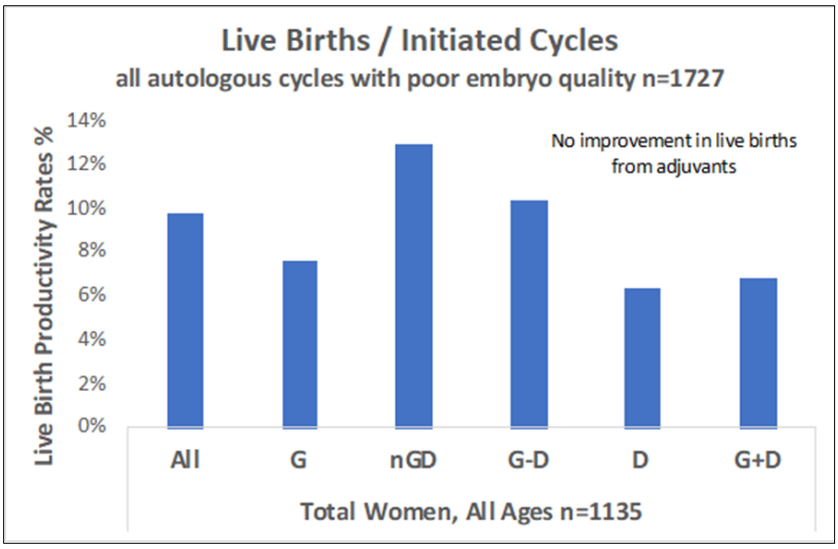

Figure 5a Bar chart depicting live birth productivity rates from the global group of 1727 IVF±ICSI treatment cycles initiated on 1135 women where embryo quality was deemed poor such that no embryos were cryopreserved. The study period was during 2011 to 2019 and embraced all age groups and ovarian reserve categories. Overall, there were 234 pregnancies from 1582 fresh ETs and no frozen ETs giving a pregnancy productivity rate per initiated cycle of 14.8%. Following early pregnancy losses, there were 170 live births generating an overall live birth productivity rate of 9.8% per initiated cycle. Neither growth hormone nor DHEA nor combinations showed any benefit over nil adjuvants

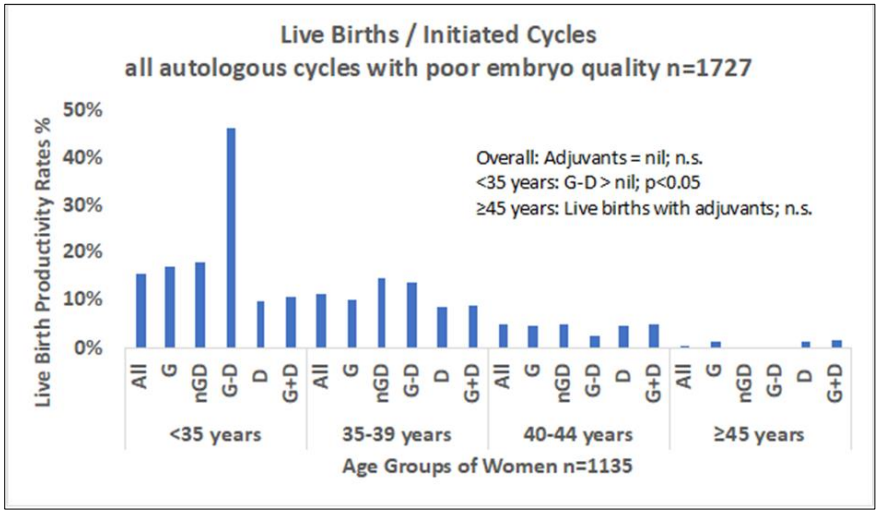

Figure 5b Bar chart depicting live birth productivity rates categorized according to age groups. No significant benefits could be shown from the use of adjuvants overall, but a marginally significant benefit was seen among women <35 years, using rGH alone (7 births from 15 initiated cycles; 46.7%; $p<0.05$). Live birth rates were very low among women aged ≥45 years; of interest each one of the 3 live births had utilised RGH alone or DHEA alone or rGH and DHEA together, respectively

3.3.3. Various POR Categories

Covering other criteria related to the Bologna POR categorization, we have further analyzed the cases of poor embryo quality with reference to the ovarian reserve groupings and the responses to ovarian stimulation represented by oocytes recovered at OPU.

Women with nil Fz and low ovarian reserve who had <5oocytes recovered

Women with low AFC groups D & E shown in Table 1c, and nil embryos cryopreserved indicate 329 women undertook 514 IVF±ICSI cycles. Relatively few oocytes (<5) were recovered in this category, with mean 2.1 oocytes, ranging from mean levels 2.0 to 2.1 across the adjuvant categories. The outcomes showed a 73.6% embryo utilization rate overall with both adjuvants showing significant effects upon the embryo utilization rate (Nil 70.7% v rGH 78.5%, DHEA 75.7% and rGH+DHEA 77.1%, $p<0.05$ for each adjuvant.). The live birth productivity rate was 4.3% overall and the rates for adjuvant categories showed no improvement by growth hormone nor DHEA (Nil 6.0% vs 4.0% and 3.4%, respectively, n.s.). Neither were there any significant findings among the age groups in this category of low ovarian reserve with few oocytes at OPU.

Women with nil Fz and high ovarian reserve but had few oocytes recovered

Women with high AFC groups A to C shown in Tables 1a and 1b, but nil embryos cryopreserved indicate 175 women undertook 206 IVF±ICSI cycles and it can be seen that few oocytes were recovered in this category, averaging 2.6 overall, ranging from mean levels 2.5 to 3.0 across the adjuvant categories. The outcomes showed a 61.6% embryo utilization rate and live birth productivity rate 11.7% overall. rGH marginally improved the embryo utilization rate (Overall 71.3% vs 58.5%; $p<0.05$). However, live birth productivity rates were not improved by growth hormone nor DHEA (Nil 15.0% vs 5.6% and 6.6%, respectively: n.s.). Neither were there any significant findings among the age groups in this category of high ovarian reserve with few oocytes recovered at OPU.

Women with nil Fz despite high ovarian reserve and high oocyte recoveries

Women with high AFC groups A to C shown in Tables 1a and 1b, and >4 oocytes recovered but nil embryos cryopreserved indicate 485 women undertook 659 IVF±ICSI cycles and adequate numbers of oocytes were recovered in this category, averaging 10.1 overall, ranging from mean levels 9.0 to 10.5 across the adjuvant categories. The outcomes showed a low 28.2% embryo utilization rate and a concomitant low live birth productivity rate 13.7% overall. Growth hormone significantly improved the embryo utilization rate across the entire age range (G 37.7% vs 30.6%, $p<0.0001$), particularly so in the younger groups of women <40 years. (Age <35 years rGH 35.3% vs nil 24.3%; $p<0.01$ and age 35-39 years G 33.8% vs nil 27.8%; $p<0.05$) as well. However, LBPR/ initiated cycle was not improved by rGH nor DHEA (Nil 16.2% vs 11.4% and 9.2%, respectively, n.s.). Neither were there any significant findings regarding live birth rates among the age groups.

Women with nil Fz and low ovarian reserve but high oocyte recoveries.

Women with low AFC groups D & E shown in Table 1c, and >4 oocytes recovered but nil embryos cryopreserved indicate 222 women undertook 291 IVF±ICSI cycles and mid-range numbers of oocytes were recovered in this category, averaging 7.7 overall, ranging from mean levels 7.0 to 8.1 across the adjuvant categories. The outcomes showed 41.8% embryo utilization, ranging from 31.5% for nil adjuvants to 47.2% for both G and G+D adjuvants and 45.0% for DHEA, these elevated rates being highly significant across the full spectrum ($P<0.0001$). Growth hormone showed significant increase in the embryo utilization rates at the younger ages (<35 years, 42.5% vs 30.2%; $p<0.01$ and 35-39 years 39.8% vs 30.6%, $p<0.05$ as did DHEA at 40.0%, $p<0.01$). However, notwithstanding these improvements in embryo utilization rates, the LBPR/ initiated cycle was not improved by growth hormone nor DHEA at any of the age groups. The live birth productivity rate was 9.4% overall with the highest rate recorded for nil adjuvants at 12.5% indicating no benefits from the adjuvants.

3.3.4. Low IGF-1 Group

Women within the low IGF-1 Group, embracing all AFC groups A to E, but nil embryos cryopreserved indicate 499 women undertook 819 IVF±ICSI cycles. A mid-range numbers of oocytes were recovered in this category, averaging 6.4 overall, ranging from mean levels 5.3 to 7.0 across the adjuvant categories. The outcomes showed a 36.5% embryo utilization rate, ranging from 31.5% for nil adjuvants to 44.3% each for both rGH and DHEA and 45.9% for the combined rGH+ DHEA ($p<0.0001$). This benefit was particularly seen in the younger groups of women <40 years. (Age <35 years rGH 43.5% vs nil 28.6%, and age 35-39 years G 39.7% vs nil 28.3%; combined $p<0.005$). However, overall LBPR/ initiated cycle was not improved by rGH or DHEA at 9.4% overall, ranging 5.7% for DHEA to 10.8% for rGH and a high of 16.6% for nil adjuvants. (Nil 12.5% vs 10.8% and 5.7%, respectively, However, the LBPR was higher for both rGH and DHEA in those women <35 years, but this proved to be an insignificant rise. In conclusion neither of the adjuvants had any benefit over nil adjuvants with respect to live birth outcomes.

3.3.5. Women with RIF (≥3 OPUs)

Women within all AFC groups A to E, but nil embryos cryopreserved indicate 358 women undertook 582 IVF±ICSI cycles and l numbers of oocytes were recovered in this category, averaging 5.7 overall, ranging from mean levels 5.1 to 6.3 across the adjuvant categories. The outcomes showed a 42.9% embryo utilization rate, ranging 38.8% for nil adjuvants to 44.8% for rGH and 46.6% for DHEA. At the younger ages <40 years, rGH showed significantly higher embryo utilization p<0.02). However, the live birth productivity rate was low at 7.2%, ranging 5.4% for DHEA to 11.1% for rGH alone vs 10.0% for nil adjuvants. These rates were not significantly different, and it is apparent that the adjuvants did not improve the live birth rates.

4. Discussion

The data presented in this 10-year retrospective study can be categorized into 2 series, firstly a global analysis of the embryology and clinical outcomes from all women undertaking autologous treatment cycles with IVF±ICSI, and secondly, a similar analysis conducted on those women who failed to achieve any cryopreserved embryos. The latter comprised a sub-set of the global series and were deemed to have poor embryo quality.

The first, global group involved 3637 treatment cycles where adjuvants rGH alone was utilized in 27.6% and DHEA was utilized in 32.5% sometimes combined with rGH. Overall oocyte numbers and the overall resultant embryo utilization rates were not improved by either of the adjuvants, albeit that older woman (in the group 40-44 years) showed a better rate with both rGH and DHEA. Nonetheless, the live birth productivity rates were not influenced by either of the adjuvants, whether used alone or in combination. In fact, the highest live birth rates ensued in those women who did not utilize adjuvants. These findings prevailed across all the age grouping and subcategories for ovarian reserve gradings. However, women within specific poor prognosis categories, namely a reduced ovarian reserve, a low IGF profile, recurrent implantation failure or low oocyte numbers at OPU each showed significant improvements in the embryo utilization rates from either rGH or DHEA or both rGH and DHEA combined. Nonetheless, these improvements also failed to translate into clinical benefits from the utilization of adjuvants as the live birth rates per initiated cycle were not improved above the rates for those women who did not use either of the adjuvants; in fact, their LPBRs were significantly higher.

The second series, concerning the influence of embryo quality was analyzed from the 47.5% of women who failed to generate any blastocysts of sufficient quality for cryopreservation; the group deemed to have poor embryo quality. This sub-set with embryos of poor quality did show significantly improved embryo utilization rate with both rGH and with DHEA as well as with combined rGH+DHEA. In keeping with the selection of poor prognosis categories, along with the fact that pregnancies were derived only from fresh transfers (by definition, no frozen embryo transfers contributed to the LBPR) the pregnancy rates were universally low in this series comprising women with poor embryo quality. However, the live birth rates remained low for each of the adjuvants and these rates were not significantly different from the nil adjuvant group which also showed similar low live birth rates.

Focusing on the respective poor prognosis categories within the second series (of poor embryo quality), embryo utilization rates were higher in each of the sub-groups utilizing adjuvants, including advanced age of the woman, those with low ovarian reserve (by AFC and AMH), those with low IGF-1 levels and those who demonstrated recurrent implantation failure. The improved embryo utilization occurred in those women using either rGH or DHEA, as well as those using both rGH+DHEA. Furthermore, although live births were not significantly increased above those in women who did not use adjuvants, the differential rates were not markedly dissimilar (unlike the first series, global group). It is possible that the treatment courses of both rGH and DHEA (6 weeks) were too short and a more prolonged treatment, in keeping with the known maturation process of the pre-ovulatory oocyte of around 6 months, might have led to improved live birth rates in those women with poor prognosis factors.

This study should be considered in follow-up to the recently reported adjuvant study which examined the use of rGH and melatonin within this population of women at PIVET. The outcomes on embryo utilization showed similar results for rGH but no significant benefit from melatonin. However, this current study does show improved embryo utilization rates from both rGH and DHEA. Neither rGH nor melatonin showed any improvement on live birth rates but we would consider that further studies are warranted for rGH and DHEA on those women with poor prognosis factors who display poor embryo quality (defined as nil embryos to cryopreservation after a single fresh embryo transfer.

Our findings on improved embryo utilization rates for women with poor embryo quality is mirrored in other recent reports elsewhere [12,15,35-39] and concurs with physiological studies showing improved oocyte competence at many levels [40-45] for rGH. The evidence for DHEA is much weaker than for rGH but our data indicates that both adjuvants

should be studied with a longer treatment regimen. Furthermore, the idea of defining adult growth hormone deficiency by dynamic testing of study subjects should also be considered. Dynamic testing applying the gold-standard insulin stimulation test, or the safer glucagon stimulation test, might assist in defining the precise group of women who could benefit from these adjuvants.

5. Conclusion

The notion that rGH, and possibly other adjuvants such as DHEA, could improve the chances of a live birth from ART treatments was predicated on studies undertaken 10-15 years ago, including several from PIVET. However, sceptics could point out that the early studies were conducted on small numbers of women, and which failed to consider the numerous variables prevalent in those years, and which could act as confounders. Furthermore, the studies failed to meet the highest standards of EBM, albeit that a satisfactory prospective RCT has yet to be presented. In the absence of the latter, this retrospective study has merit due to the large number of women and treatment cycles studied, with data being compiled in a validated database in real-time. The idea of randomization was not feasible given that the setting is one involving private medical practice where women paid a large proportion of the costs for their treatment cycles and the ensuing IVF±ICSI procedures. Furthermore, they covered the entire cost of the adjuvants. The selection of adjuvant was considered by the patient and her personal clinician who continued to manage the woman through her work-up, her treatments and her post-operative reviews. The quality of the information applied in these situations was dependent upon that available in the medical and scientific literature at the time of consultation. That is now likely to change given the clearer, albeit disappointing, picture emerging from these two very recent studies from PIVET. This retrospective study accords with our recent prospective study showing similar findings that, although rGH and DHEA can improve embryo utilization rates in women who generate embryos of poor quality, there is no significant improvement in live birth rates. Nonetheless, we believe further studies with longer treatment courses should be undertaken along with the consideration of dynamic testing of some women, with definable poor prognosis, for potential adult growth hormone deficiency.

Compliance with ethical standards

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Disclosure of conflict of interest

The entire project has been funded internally at PIVET without any external or commercial contributions. The authors declare no conflict 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 recently, 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. Clinicians SS, MS, SG and PR have each been involved in recruiting patients and counselling them with respect to the use of adjuvants. 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 have read and agreed to its content.

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