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Applying growth hormone as an adjuvant to correct poor prognosis outcomes in IVF: Study 1 compares melatonin

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Abstract

This retrospective study examines the influence of recombinant growth hormone (rGH) and melatonin 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 4.0 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 melatonin showed any benefit on these rates, in fact, those women with nil adjuvants showed the highest live birth rate per initiated cycle (42.0% overall: $p < 0.0001$, and 55.3% for the youngest group: $p < 0.001$). Embryo utilization was increased marginally by rGH in those women aged 40-44 years who had high ovarian reserve ($p < 0.05$), but this benefit did not translate into any improvement in the live birth rate. 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 rGH, but not with melatonin. Even so, live birth rates were not improved by either of the adjuvants.

Keywords: In vitro fertilization [IVF]; Intracytoplasmic sperm injection [ICSI]; Recombinant growth hormone [rGH]; Melatonin; Embryo utilization; Live birth productivity rate

1. Introduction

Although assisted reproductive technology (ART) has earned 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 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-Müllerian 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 the part of the underlying reason for women attending ART facilities. Furthermore,

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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. Following an encouraging report in 2005 on the use of recombinant manufactured growth hormone; rGH as an adjuvant for older women undergoing assisted reproduction [15], 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 [16] but improvement in live birth rates is reported in under 50% and those studies all display design weaknesses with problematic confounding variables [17,18]. To minimise the latter, we present a 10-year study period from our pioneer Australian facility practising 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.

2. Material and methods

2.1. Study setting

Table 1 One of the PIVET FSH-dosing Algorithms which covers for 12.5 IU and 25 IU increments of FSH up to a maximum 450 IU

		Gonal-F, Puregon & Elonva Desk Chart																																			
AMH	>30 pm/L	25-29.9 pm/L					20-24.9 pm/L					15-19.9 pm/L					10-14.9 pm/L					5-9.9 pm/L					< 5.0 pm/L										
AFC*	A++ (≥ 40 follicles)	A+ (30-39 follicles)					A (20-29 follicles)					B (13-19 follicles)					C (9-12 follicles)					D (5-8 follicles)					E (≤4 follicles)										
BMI	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	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 (years)	20	37.5	37.5	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	100.0	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	187.5	200.0	200.0		
	21	37.5	37.5	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	100.0	100.0	100.0	112.5	112.5	125.0	125.0	137.5	137.7	137.7	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	
	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	100.0	100.0	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	187.5	200.0	200.0	212.5	212.5	
	23	50.0	50.0	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5	100.0	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	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5
	24	50.0	50.0	62.5	62.5	62.5	75.0	75.0	75.0	87.5	87.5	100.0	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	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	
	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	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	
	26	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	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5		
	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	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	
	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	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0		
	29	75.0	75.0	75.0	87.5	87.5	87.5	100.0	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	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	
	30	75.0	75.0	75.0	87.5	87.5	100.0	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	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0		
	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	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0		
	32	87.5	87.5	87.5	100.0	100.0	100.0	112.5	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	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	
	33	87.5	87.5	87.5	100.0	100.0	100.0	112.5	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	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	
	34	87.5	87.5	100.0	100.0	112.5	112.5	112.5	125.0	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0		
	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	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0	
	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	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0		
	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	187.5	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0	350.0	350.0	375.0	375.0	
	38	112.5	112.5	125.0	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0	350.0	350.0	375.0	375.0		
	39	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	187.5	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0	350.0	350.0	375.0	375.0	400.0	400.0	
40	112.5	112.5	125.0	137.5	137.5	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0	350.0	350.0	375.0	375.0	400.0	400.0		
41	125.0	125.0	137.5	137.5	150.0	150.0	162.5	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0	350.0	350.0	375.0	375.0	400.0	400.0		
42	125.0	125.0	137.5	150.0	150.0	162.5	162.5	175.0	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	325.0	325.0	350.0	350.0	375.0	375.0	400.0	400.0			
43	125.0	137.5	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	312.5	312.5	325.0	325.0	350.0	350.0	375.0	375.0	400.0	400.0	425.0	425.0		
44	137.5	137.5	150.0	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	312.5	312.5	325.0	325.0	350.0	350.0	375.0	375.0	400.0	400.0	425.0	425.0	450.0	450.0	
45	150.0	150.0	162.5	162.5	175.0	175.0	187.5	187.5	200.0	200.0	212.5	212.5	225.0	225.0	237.5	237.5	250.0	250.0	275.0	275.0	300.0	300.0	312.5	312.5	325.0	325.0	350.0	350.0	375.0	375.0	400.0	400.0	425.0	425.0	450.0	450.0	

Increased FSH and Smokers

- Where FSH is less than 8 IU/L, with no history of smoking, use values as shown
– Smokers move two columns to the right
- Where FSH is between 8 & 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

Oocyte Donors

- Aiming for 10-12 oocytes, move four columns to the right
- Consider GnRH Agonist trigger if >10 follicles e.g., Tryptorelin 100 µg x2

12.5 IU increments suit Gonal-F Pen
 25 IU increments also suits Puregon Pen
 Elonva – 1 x 100µg for wt ≤60kg
 1 x 150µg for wt >60kg

Version 12.1 3 April 2017

Following a 4-year period of training in London during the years 1976-1980, lead author JLY established the PIVET programme 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 [19]. 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 [20]. Since January 2011, the ART programme at PIVET has been characterised by three important developments, firstly, encouraging amenable ART-naïve women to undertake a preliminary Assessment Cycle [AC] [21]; secondly, utilising the well described PIVET dosing-algorithms for ovarian stimulation [22,23,24], 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 [25].

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 [26]. 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 (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 [27]); and poor-quality embryos resulting in zero blastocysts available or suitable for cryopreservation (nil frozen; Fz) [28]. These factors have been analyzed alone 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). Melatonin was chosen by 676 women (28.5%) in 1027 treatment cycles (28.2%) (Figure 1). Both GH and Melatonin were used in combination by 422 women (17.8%) in 635 treatment cycles (17.5%). The use of DHEA is the subject of a second related report (Study 2).

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 requirements for evidence-based medicine; EBM [12,29].

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 [30] with potential, albeit unproven benefits in IVF.

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 μ M). 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.

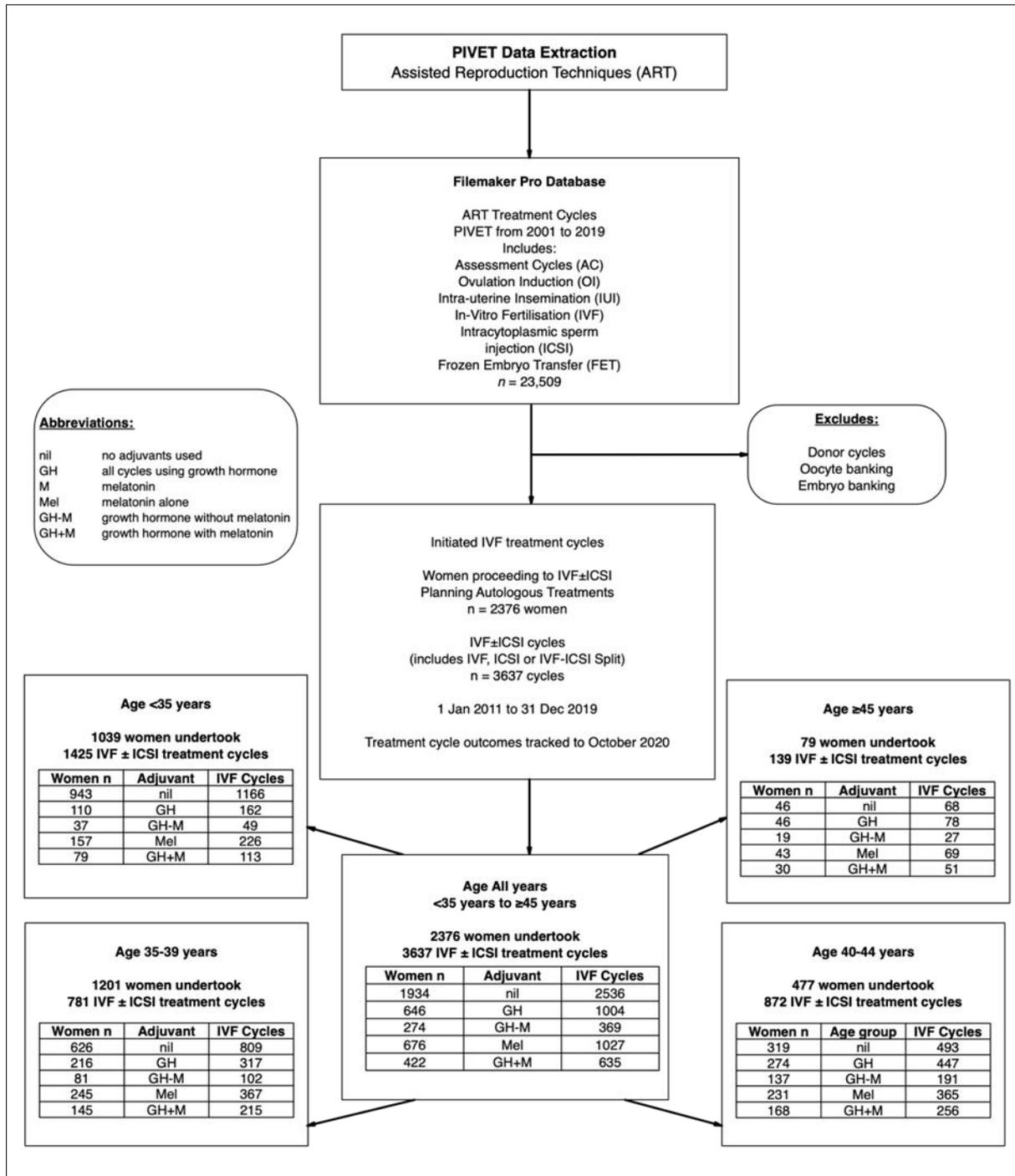


Figure 1 Flow diagram showing the derivation of the women who proceeded to IVF ± rGH adjuvant treatment cycles after undertaking an assessment cycle

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 [21].

2.3.2. Cancelled cycles

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 [21]. 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 (ref). 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 ($O Ut^n \%$)

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 pm/L and/or P4 >600 nm/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 ($E Ut^n \%$)

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 [21] means that $\sim 85\%$ 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 [28]. 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 [28].

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 [31]. 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 noted in the Tables of Outcomes, nonetheless.

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 β hCG 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 β HCG 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 [21]. 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 %)

Livebirth 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 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 [31]. Each delivery is counted as one livebirth, 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 [19]. Furthermore, PIVET records twinning rates at under 1.0% with nil triplets or higher order multiples in recent years [10]. 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) [32] 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 analysed 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, are listed here for clearer reference.

Adj	adjuvants
Canc ⁿ %	cancellation rate
O	oocyte/s
OPU	oocyte pick-up procedure
O Ut ⁿ %	oocyte utilization rate
E Ut ⁿ %	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
EPLs %	miscarriage rate (early pregnancy losses from week 5)
LBP%/In	live birth (L/B) productivity rate per initiated cycle
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
PIVET	registered acronym from programmed <u>IVF</u> & <u>ET</u>

3. Results

3.1. Global outcomes

The data reported in this Study 1 focuses on the adjuvants rGH and Melatonin deferring the consideration of DHEA for a second report as Study 2. The key laboratory outcome is represented by the E Utⁿ % and the key clinical outcome is represented by the Live birth pregnancy productivity rate per initiated IVF±ICSI treatment cycle (LBP % / In). The overall outcomes for autologous treatment cycles across all age groups are summarized in Table 2.

It can be seen that the mean embryo utilization ranges tightly at 49% to 51%, 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).

Notwithstanding the differences in rate ranges, at all ages the embryo utilization rate is not significantly increased by the use of rGH or melatonin, neither alone nor in combination [Figure 2a].

In keeping with this observation, it can be seen that the live birth rate is also not improved by the adjuvants, neither alone nor in combination [Figure 2b].

Table 2 Embryology and clinical outcomes from the global group of 3637 IVF±ICSI treatment cycles on 2376 women during 2011 to 2019 embracing all age groups and ovarian reserve categories

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E						
Ages	Totals: All ages <35 years through to ≥45 years					
Adjuvant	All	G	nGM	G -M	M	G +M
Cycles (n)	3637	1004	2536	369	1027	635
Women (n)	2376	646	1934	274	676	422
Cancelln %	0.3	0.5	0.2	0.3	0.4	0.6
O/OPU (n)	9.2	6.7	9.9	6.3	7.3	6.9
O Ut ⁿ %	26.0	25.1	26.6	26.6	24.6	24.3
E Ut ⁿ %	48.5	49.7	48.5	50.6	48.2	49.1
E Ut ⁿ Stats	adjuvants or combinations compared to nil adjuvants (n.s.)					
E Fz %	30.5	21.2	31.9	17.7	24.9	23.1
PP/ In %	46.4	22.9	53.5	22.0	28.2	23.5
PP/ OPU %	46.6	23.0	53.7	22.0	28.3	23.6
PP/ ET %	61.8	33.4	68.4	29.6	41.3	36.0
EPLs %	23.7	29.1	21.6	22.2	31.0	32.9
LBP/ In %	35.4	16.2	42.0*	17.1	19.5	15.7
LBP/ In Stats	*nil adjuvants better than G, M or combinations (p<0.0001)					
LBP/ OPU %	35.6	16.3	42.1	17.1	19.6	15.8
LBP/ET %	47.2	23.7	53.6	23.0	28.5	24.2

Table 2a Embryology and clinical outcomes from the younger groups of women comprising 1039 who had 1425 IVF±ICSI treatment cycles during 2011 to 2019 embracing all ovarian reserve categories

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E												
Ages	<35 years						35-39 years					
Adjuvant	All	G	nGM	G -M	M	G +M	All	G	nGM	G -M	M	G +M
Cycles (n)	1425	162	1166	49	226	113	1201	317	809	102	367	215
Women (n)	1039	110	943	37	157	79	781	216	626	81	245	145
Cancell ⁿ %	0.1	0.0	0.2	0.0	0.0	0.0	0.4	0.3	0.4	0.0	0.3	0.5
O/OPU (n)	11.1	8.8	11.4	7.7	9.4	9.3	9.4	7.5	10.1	7.7	7.8	7.4
O Ut ⁿ %	26.9	23.9	27.5	24.3	23.8	23.8	25.3	23.9	25.7	23.9	24.5	23.8
E Ut ⁿ %	49.3	48.5	49.5	46.5	47.3	49.3	47.0	45.6	47.0	44.4	46.6	46.3
E Ut ⁿ Stats	adjuvants or combinations compared to nil adjuvants (n.s.)											
E Fz %	35.7	30.2	36.3	25.3	30.4	32.1	29.6	20.2	31.0	16.8	25.0	22.0
PP/ In %	69.3	44.4	73.9	55.1	45.6	39.8	45.3	30.9	49.6	36.3	35.1	28.4
PP/ OPU %	69.4	44.4	74.1	55.1	45.6	39.8	45.5	31.0	49.8	36.3	35.2	28.5
PP/ ET %	88.0	67.9	92.1	81.8	61.7	61.6	60.1	42.6	63.0	43.5	50.6	42.1

EPLs %	20.3	23.6	19.8	22.2	23.3	24.4	23.9	29.6	20.0	18.9	32.6	36.1
LBP/ In %	55.2	34.0	59.3*	42.9	35.0	30.1	34.5	21.8	39.7*	29.4	23.7	18.1
LBP/ In Stats	<i>*nil adjuvants better than G, M or combinations (p<0.001)</i>											
LBP/ OPU %	55.3	34.0	59.4	42.9	35.0	30.1	34.6	21.8	39.8	29.4	23.8	18.2
LBP/ET %	70.1	51.9	73.8	63.6	47.3	46.6	45.7	30.0	50.4	35.3	34.1	26.9

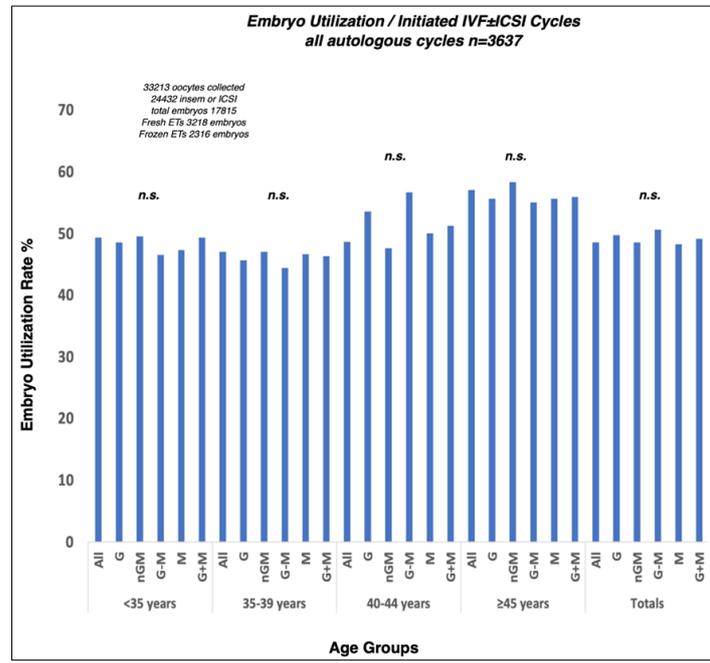


Figure 2a Bar chart depicting embryo utilization rates from the global group of 3637 IVF±ICSI treatment cycles undertaken on 2376 women during 2011 to 2019 embracing all age groups and ovarian reserve categories

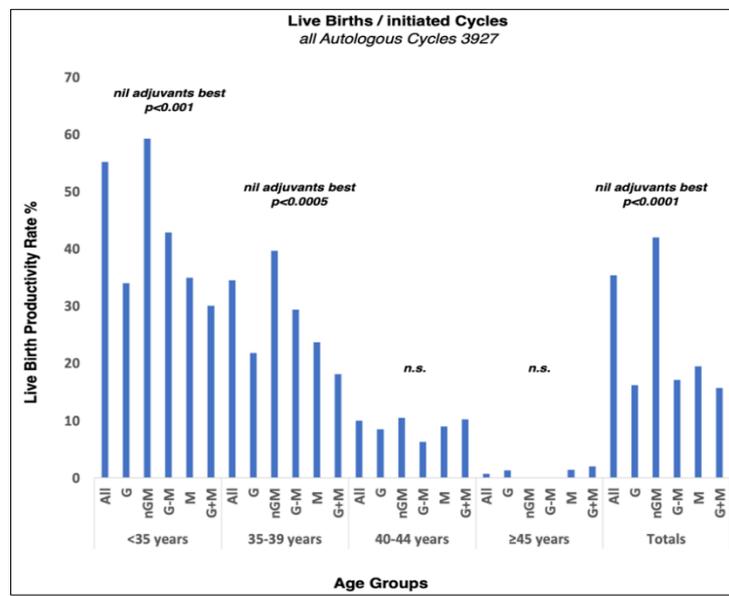


Figure 2b Bar chart depicting live birth productivity rates from the global group of 3637 IVF±ICSI treatment cycles undertaken on 2376 women during 2011 to 2019 embracing all age groups and ovarian reserve categories

In fact, in this global dataset 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 under age 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. There were no significant differences demonstrated with or without adjuvants for those women ≥ 40 years.

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

Autologous IVF±ICSI Cycles, during 2011-2019: all AFC Groups A to E												
Ages	40-44 years						≥45 years					
Adjuvant	All	G	nGM	G -M	M	G +M	All	G	nGM	G -M	M	G +M
Cycles (n)	872	447	493	191	365	256	139	78	68	27	69	51
Women (n)	477	274	319	137	231	168	79	46	46	19	43	30
Cancell ⁿ %	0.6	0.9	0.2	0.5	0.8	1.2	0.0	0.0	0.0	0.0	0.0	0.0
O/OPU (n)	6.5	5.8	6.8	5.4	6.1	6.1	4.1	4.1	3.9	4.3	4.3	3.9
O Ut ⁿ %	24.9	26.5	24.9	29.3	24.9	24.6	29.2	28.5	30.3	28.7	28.7	28.4
E Ut ⁿ %	48.6	53.5	47.6	56.6	50.0	51.2	57.0	55.6	58.3	55.0	55.6	55.9
E Ut ⁿ Stats	<i>adjuvants or combinations compared to nil adjuvants (n.s.)</i>											
E Fz %	18.5	18.1	17.2	15.5	20.6	19.9	16.4	13.6	15.8	18.3	17.0	10.8
PP/ In %	17.8	13.2	19.1	8.9	15.6	16.4	1.4	1.3	1.5	0.0	1.4	2.0
PP/ OPU %	17.9	13.3	19.1	8.9	15.7	16.6	1.4	1.3	1.5	0.0	1.4	2.0
PP/ ET %	25.0	19.3	25.4	12.1	23.7	25.3	2.4	2.2	2.3	0.0	2.6	3.3
EPLs %	43.9	35.6	44.7	29.4	42.1	38.1	50.0	0.0	100	0.0	0.0	0.0
LBP/ In %	10.0	8.5	10.5	6.3	9.0	10.2	0.7	1.3	0.0	0.0	1.4	2.0
LBP/ In Stats	<i>adjuvants or combinations compared to nil adjuvants (n.s.)</i>											
LBP/ OPU %	10.0	8.6	10.6	6.3	9.1	10.3	0.7	1.3	0.0	0.0	1.4	2.0
LBP/ET %	14.0	12.4	14.1	8.6	13.7	15.7	1.2	2.2	0.0	0.0	2.6	3.3

3.1.1. Subsets of global data

From this global dataset, further analysis is categorized for subsets of various clinical prognostic criteria with key embryological information and clinical outcomes summarized. The full data has generated a large number of Tables and Figures which, being outside the limits for this article, are not shown; however, they have all been prepared and are available at request from the corresponding author.

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.

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. These groups range from the highest A group with AFC numbers > 20 antral follicles (with sub-classifications A, A+ and A++) progressively downwards to the lowest Group E with < 5 antral follicles. 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). A low ovarian reserve embraces Groups D and E, meaning < 9 antral follicles).

Total AFC Groups A to E

Table 2 summarizes the outcomes of 2376 women who undertook 3637 IVF±ICSI treatment cycles during the completed years 2011 to 2019. Included are women from all the AFC Groups A (with ≥ 20 antral follicles and/or AMH levels >20 pmol/l) through to E (with ≤ 4 antral follicles and/or AMH <5 pmol/l). An average of 9.2 oocytes were recovered at OPU overall and mean numbers ranged from 6.3 to 9.9 across the adjuvant groupings with no significant differences demonstrated. Overall, 26.0% of oocytes contributed to the formation of usable embryos designated as the embryo utilization rate which was 48.5%. There were no significant differences in embryo utilization across the various adjuvant groupings. The live birth productivity rate per initiated treatment cycle showed an average of 35.4% across all ages and adjuvant groupings but neither rGH, Melatonin or combinations showed any benefit over the nil adjuvant group. In fact, as shown in Figure 2, the nil adjuvant group displayed the highest live birth rate (LBP/Initiated cycle 42.0%) being significantly higher than each of the adjuvant groupings ($p < 0.0001$).

Sub-categorizing the data from Table 2 into that for the younger women (<40 years, Table 2a) and the older women (≥ 40 years, Table 2b) shows interesting differences. The mean oocyte numbers for the younger women ranged from 11.1 for those <35 years down to 9.4 for those aged 35-39 years, while the mean oocyte numbers for the older women ranged 6.5 for those aged 40-44 years down to 4.0 for those aged ≥ 45 years, the differences between younger and older being highly significant ($p < 0.0001$). These differences occurred despite the older women receiving higher FSH dosing according to the PIVET Algorithm (to a maximum 450 IU/day). 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 particular age group (Figure 2a). The live birth productivity rates were significantly higher for the younger women (55.3% down to 34.5%) compared to the older women (10.0% down to 0.7%, $p < 0.0001$). However, 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 (Figure 2b, $p < 0.001$).

High Ovarian Reserve; represented by high AFC and AMH

Women with high AFC (and AMH) groups A to C were analyzed as a subset. The data show 1606 women undertook 2312 IVF±ICSI cycles showing that an average 11.1 oocytes / OPU were recovered overall. The mean levels ranged 9.1 to 11.6 across the adjuvant categories without any statistical significance. The resultant embryo utilization rate overall was 46.8% and live birth productivity rate was 43.3%. Adjuvants did not improve embryo utilization overall neither across the age groups, other than Growth Hormone marginally in the 40-44 years group (44.3% vs 39.5%, $p < 0.05$). Live birth productivity rates were significantly highest for nil adjuvants rather than either Growth Hormone or Melatonin (49.2% vs 22.5% and 25.3% respectively; $p < 0.0001$).

Low Ovarian Reserve; represented by low AFC and AMH

Women with low AFC (& AMH) groups D & E were analyzed as a subset. The data show 710 women undertook 1262 IVF±ICSI cycles showing that an average 5.6 oocytes / OPU were recovered overall. The mean levels ranged 4.5 to 6.1 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 53.0% nil adjuvants to 60.0%, $p < 0.0001$), most prominently in the 40-44 years group. However, live birth productivity rates were significantly highest for nil adjuvants rather than Growth Hormone or Melatonin (25.7% vs 10.8% and 11.9% respectively; $p < 0.0001$).

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

Women with low IGF-1 levels (<21 nmol/L) were analyzed as a subset. The data show 1037 women undertook 1755 IVF±ICSI cycles showing that an average 9.8 oocytes / OPU were recovered overall. The mean levels ranged 7.1 to 10.9 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 Growth hormone or Melatonin, neither the combination of adjuvants. Furthermore, live birth productivity rates were significantly highest for nil adjuvants rather than Growth Hormone or Melatonin (46.6% vs 14.2% and 19.6% respectively; $p < 0.0001$).

3.2.3. Recurrent Implantation Failure

Women with ≥ 3 OPU's without a pregnancy success were analyzed as a subset. The data show 556 women undertook 982 IVF±ICSI cycles showing that an average 7.6 oocytes / OPU were recovered overall. The mean levels ranged 6.4 to 7.7 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 Growth hormone or Melatonin, neither with the combination. Additionally, there were no significant differences in live birth productivity rates for adjuvants Growth Hormone or Melatonin vs nil adjuvants (n.s.)

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).

3.2.5. High oocyte numbers: ≥ 5 oocytes recovered

Women with ≥ 5 oocytes at OPU were analyzed as a subset. The data show 1970 women undertook 2723 IVF \pm ICSI cycles showing that an average 11.4 oocytes / OPU were recovered overall. The mean levels ranged 9.7 to 11.9 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 Growth hormone or Melatonin, neither the combination. Adjuvants did not improve the live birth productivity rates, on the contrary nil adjuvants had significantly higher rates than either Growth Hormone or Melatonin (49.4% vs 23.7% and 27.1% respectively, $p < 0.0001$).

3.2.6. Low numbers: <5 oocytes recovered

Women with <5 oocytes at OPU were analyzed as a subset. The data show 597 women undertook 886 IVF \pm 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 Growth hormone 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 Growth Hormone or Melatonin 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 Growth Hormone or Melatonin (24.5% vs 9.3% and 6.2% respectively; $p < 0.01$).

3.3. Embryo quality

The prognosis for IVF has mainly been projected from the Bologna Criteria focussing 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 analysing 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 Frozen; 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 frozen.

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

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

These indicate 1135 women, being 47.8% of the study group, undertook 1727 IVF \pm ICSI cycles showing that an average of 6.3 oocytes / OPU were recovered overall. The mean levels ranged 5.1 to 6.7 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 37.3% for nil adjuvants to 50.3% for rGH adjuvant, a rate which is significantly increased ($p < 0.001$) [Figure 3a].

Melatonin showed no similar benefit. 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 [Figure 3b].

Table 3 Embryology and clinical outcomes from the sub-series of 1135 women who had 1727 IVF±ICSI treatment cycles during 2011 to 2019 embracing all age groups and ovarian reserve categories, but whose embryos were classified as poor quality with nil reaching cryopreservation

Autologous IVF±ICSI Cycles, during 2011-2019: nil embryos cryopreserved (Fz)						
Ages	Totals: All ages <35 years through to ≥45 years					
Adjuvant	All	G	nGM	G -M	M	G +M
Cycles (n)	1727	668	1092	266	596	402
Women (n)	1135	457	826	201	419	288
Cancell ⁿ %	0.7	0.7	0.5	0.4	0.7	1.0
O/OPU (n)	6.3	5.1	6.7	4.8	5.3	5.2
O Ut ⁿ %	14.7	20.3	13.7	22.8	17.8	18.7
E Ut ⁿ %	39.3	47.3*	37.3	50.3*	43.1	45.3
E Ut ⁿ Stats	<i>*G significantly higher compared to nil adjuvants (p<0.001)</i>					
E Fz %	0.0	0.0	0.0	0.0	0.0	0.0
PP/ In %	13.5	10.8	15.3	10.2	10.4	11.2
PP/ OPU %	13.6	10.9	15.4	10.2	10.5	11.3
PP/ ET %	19.1	15.3	21.1	13.8	14.8	16.4
EPLs %	27.4	29.2	24.0	18.5	32.3	35.6
LBP/ In %	9.8	7.6	11.6	8.3	7.0	7.2
LBP/ In Stats	<i>Adjuvants (G, M ± combinations) vs nil adjuvants (n.s.)</i>					
LBP/ OPU %	9.9	7.7	11.7	8.3	7.1	7.3
LBP/ET %	13.8	10.9	16.1	11.3	10.0	10.5

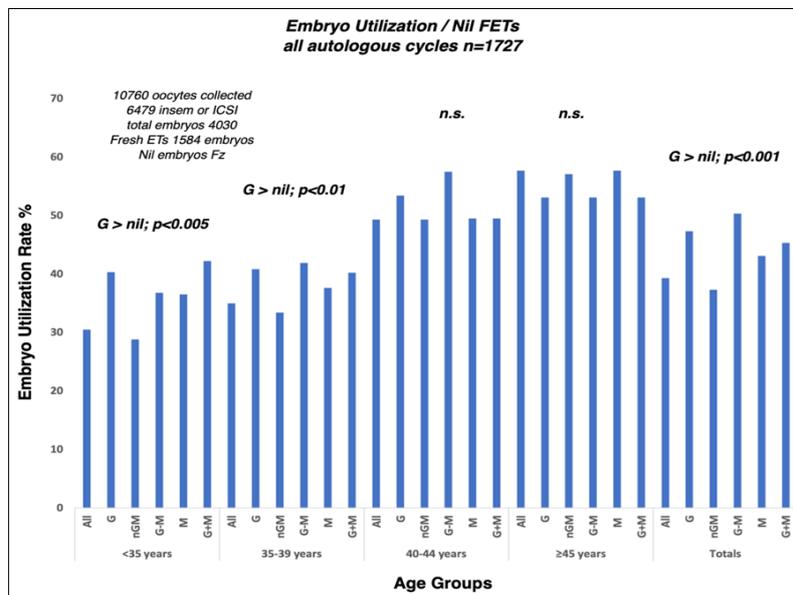


Figure 3a Bar chart depicting embryo utilization rates from the sub-series group of 1135 women who undertook 1727 IVF±ICSI treatment cycles during 2011 to 2019 embracing all age groups and ovarian reserve categories, but whose embryos were classified as poor quality with nil reaching cryopreservation

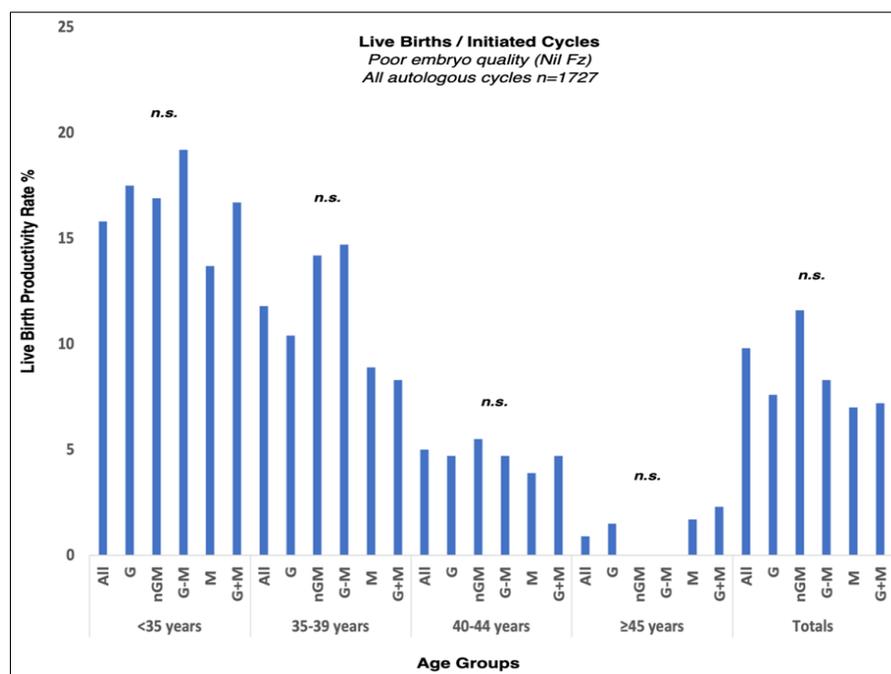


Figure 3b Bar chart depicting live birth rates from the sub-series group of 1135 women who undertook 1727 IVF±ICSI treatment cycles during 2011 to 2019 embracing all age groups and ovarian reserve categories, but whose embryos were classified as poor quality with nil reaching cryopreservation

Live birth outcomes were low across the dataset ranging 7.1% for Melatonin to 7.7% for rGH and 11.7% for nil adjuvants; these rates being not significantly different.

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

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.

Younger women

The outcomes for younger women (aged <40 years) who had zero embryos Fz are depicted in Table 9a with Embryo utilization rates shown in Figure 3a.

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 GH, particularly for the youngest women, in the <35 years age group (40.3% vs 28.8%; $p < 0.01$). Melatonin showed no significant benefit. Live birth productivity rates are shown in Table 9a along with Figure 9b. Notwithstanding the rGH benefit on embryo utilization, no beneficial effect was demonstrated on live birth rates (rGH 17.5%, M 13.7% and nil adjuvants 16.9% for <35 years; n.s.)

Older women

The outcomes for older women (aged ≥40 years) who had zero embryos Fz are depicted in Table 9b with Embryo utilization rates shown in Figure 3a.

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 not significantly higher for rGH in either of the two higher age groupings. Melatonin also showed no significant benefit. 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%, M 3.9% and nil adjuvants 5.5% for the younger group; n.s.).

Table 3a Embryology and clinical outcomes from the younger groups of women comprising 352 who had 476 IVF±ICSI treatment cycles during 2011 to 2019 embracing all ovarian reserve categories, but whose embryos were classified as poor quality with nil reaching cryopreservation

Autologous IVF±ICSI Cycles, during 2011-2019: nil embryos cryopreserved (Fz) Age <40 years												
Ages	<35 years						35-39 years					
Adjuvant	All	G	nGM	G -M	M	G +M	All	G	nGM	G -M	M	G +M
Cycles (n)	476	80	360	26	102	54	549	201	332	68	203	133
Women (n)	352	57	296	19	79	42	372	146	263	56	140	95
Cancell ⁿ %	0.4	0.0	0.6	0.0	0.0	0.0	0.9	0.5	0.9	0.0	0.5	0.8
O/OPU (n)	7.8	5.4	8.1	4.9	6.3	5.6	6.8	5.9	7.2	6.1	5.9	5.9
O Ut ⁿ %	9.6	14.8	9.2	16.5	13.1	14.1	13.3	18.0	12.2	18.9	16.3	17.4
E Ut ⁿ %	30.5	40.3*	28.8	36.8	36.5	42.2*	35.0	40.8	33.4	41.9	37.6	40.2
E Ut ⁿ Stats	<i>G adjuvant better than nil or M adjuvants (p<0.01.)</i>											
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	20.6	19.2	17.6	20.4	14.8	14.9	16.3	17.6	12.8	13.5
PP/ OPU %	19.6	20.0	20.7	19.2	17.6	20.4	14.9	15.0	16.4	17.6	12.9	13.6
PP/ ET %	28.4	31.4	29.7	31.3	24.0	31.4	20.6	20.1	22.3	22.6	17.8	18.8
EPLs %	19.4	12.5	17.6	0.0	22.2	18.2	19.8	30.0	13.0	16.7	30.8	38.9
LBP/ In %	15.8	17.5	16.9	19.2	13.7	16.7	11.8	10.4	14.2	14.7	8.9	8.3
LBP/ In Stats	<i>adjuvants or combinations compared to nil adjuvants (n.s.)</i>											
LBP/ OPU %	15.8	17.5	17.0	19.2	13.7	16.7	11.9	10.5	14.3	14.7	8.9	8.3
LBP/ET %	22.9	27.5	24.5	31.3	18.7	25.7	16.5	14.1	19.4	18.9	12.3	11.5

Table 3b Embryology and clinical outcomes from the older groups of women comprising 339 who had 585 IVF±ICSI treatment cycles during 2011 to 2019 embracing all ovarian reserve categories, but whose embryos were classified as poor quality with nil reaching cryopreservation

Autologous IVF±ICSI Cycles, during 2011-2019: nil embryos cryopreserved (Fz) Age ≥40 years												
Ages	40-44 years						≥45 years					
Adjuvant	All	G	nGM	G -M	M	G +M	All	G	nGM	G -M	M	G +M
Cycles (n)	585	319	344	148	232	171	117	68	56	24	59	44
Women (n)	339	210	229	109	159	121	72	44	38	17	41	30
Cancell ⁿ %	0.9	1.3	0.3	0.7	1.3	1.8	0.0	0.0	0.0	0.0	0.0	0.0
O/OPU (n)	5.1	4.7	5.3	4.5	4.8	4.9	3.5	3.6	3.4	3.3	3.5	3.8
O Ut ⁿ %	21.3	23.0	21.9	26.6	20.5	20.0	26.0	24.6	25.1	21.5	27.3	26.1
E Ut ⁿ %	49.3	53.4	49.3	57.5	49.5	49.5	57.7	53.1	57.1	53.1	57.7	53.1
E Ut ⁿ Stats	<i>adjuvants or combinations compared to nil adjuvants (n.s.)</i>											
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	11.0	6.8	7.3	8.8	1.7	1.5	1.8	0.0	1.7	2.3
PP/ OPU %	10.0	7.9	11.1	6.8	7.4	8.9	1.7	1.5	1.8	0.0	1.7	2.3
PP/ ET %	13.4	10.9	14.5	8.8	10.4	12.8	2.7	2.5	2.7	0.0	2.8	3.7
EPLs %	50.0	40.0	50.0	30.0	47.1	46.7	50.0	0.0	100	0.0	0.0	0.0
LBP/ In %	5.0	4.7	5.5	4.7	3.9	4.7	0.9	1.5	0.0	0.0	1.7	2.3
LBP/ In Stats	<i>adjuvants or combinations compared to nil adjuvants (n.s.)</i>											
LBP/ OPU %	5.0	4.8	5.5	4.8	3.9	4.8	0.9	1.5	0.0	0.0	1.7	2.3
LBP/ET %	6.7	6.5	7.3	6.2	5.5	6.8	1.4	2.5	0.0	0.0	2.8	3.7

3.3.3. Various POR Categories

Covering other criteria related to the Bologna POR categorisation, 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 few oocytes recovered

Women with low AFC groups D & E and nil embryos cryopreserved were analyzed as a subset. The data show 490 women undertook 819 IVF±ICSI cycles. It can be seen that relatively few oocytes (<5) were recovered in this category, averaging 4.0 overall, ranging from mean levels 3.6 to 4.3 across the adjuvant categories. The outcomes showed a 53.6% embryo utilisation rate overall. Adjuvants showed no effect on the embryo utilization rate (Nil 52.4% v Growth Hormone 59.8% and Melatonin 55.6%, n.s.). The live birth productivity rate was 6.3% overall and the rates for adjuvant categories showed no improvement by Growth Hormone or Melatonin (Nil 7.1% vs 6.3% and 5.3%, respectively, n.s.). Neither were there any significant findings among the age groups.

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

Women with high AFC groups A to C but nil embryos cryopreserved were analyzed as a subset. The data show 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 or Melatonin (Nil 15.0% vs 5.6% and 6.6%, respectively: n.s.). Neither were there any significant findings among the age groups.

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

Women with high AFC groups A to C, and >4 oocytes recovered but nil embryos cryopreserved were analyzed as a subset. The data show 486 women undertook 660 IVF±ICSI cycles and adequate numbers of oocytes were recovered in this category, averaging 10.1 overall, ranging from mean levels 9.1 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 13.8% 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, live birth productivity rates/ initiated cycle were not improved by rGH or Melatonin (Nil 14.2% vs 11.4% and 9.9%, 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 and >4 oocytes recovered but nil embryos cryopreserved were analyzed as a subset. The data show 725 women undertook 968 IVF±ICSI cycles and adequate oocytes were recovered in this category, averaging 9.4 overall, ranging from mean levels 8.5 to 9.5 across the adjuvant categories. The outcomes showed 32.0% embryo utilization and live birth productivity rate 12.6% overall. Growth hormone showed significant increase in the embryo utilization rate at the younger ages (<35 years, 35.3% vs 24.3%; $p < 0.01$ and 35-39 years 33.8% vs 27.8%; $p < 0.05$ as well as across the entire age range; totals 37.7% vs 30.6%; $p < 0.0001$). However, the live birth productivity rates/ initiated cycle was not improved by Growth Hormone or Melatonin (totals: nil 14.2% vs 11.4% and 9.9%, respectively; n.s.), neither at any of the age groups.

4. Discussion

The data presented in this 10-year retrospective study can be categorised 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 melatonin was utilized in 28.2% 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 marginally better rate with rGH. Furthermore, 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. Furthermore, women within specific poor prognosis categories, namely a low IGF profile, recurrent implantation failure and low oocyte numbers also failed to show any improvements in either embryology data or clinical outcomes from the utilization of adjuvants, neither rGH nor melatonin.

The second series, concerning the influence of embryo quality was analyzed from the 47.8% of women who failed to generate any blastocysts of sufficient quality for cryopreservation. This sub-set with embryos of poor quality did show a significantly improved embryo utilization rate with rGH, but not with melatonin. However, the live birth rate remained low for each of the adjuvants but were not significantly different from the nil adjuvant group. Furthermore, overall, there were no significant differences among the adjuvant categories for each of the age groupings. Examining age-related outcomes and the influence of ovarian reserve categories with respect to whether low or high oocyte numbers were recovered, one group, namely those women who had a high ovarian reserve and who had high numbers of oocytes retrieved, did show significant improvement in embryo utilization with rGH, but not with melatonin. The rGH benefit was seen across all the age groups for those women categorised with either high or low ovarian reserve but who, nonetheless, had high oocyte numbers retrieved. In fact, the benefit was most marked among the younger groups of women. However, this improvement did not translate to any benefit in the live birth rates which remained low and unimproved across the age groups regardless of any adjuvant use. Of interest those women who had the lowest number of oocytes retrieved (only one or two) had significantly higher embryo utilization rates, but again, these were not influenced by either of the adjuvants. Furthermore, live birth rates were not improved, remaining low across the age groupings, and not influenced by the adjuvants. The highest embryo utilization seen with the lowest egg numbers is likely due to two factors – one bears on the low ovarian reserve such that those women who responded to FSH stimulation generated very few small follicles which often contain immature oocytes, the second relates to the fact that such women have their “best” embryo transferred at the early cleavage stage when embryo quality measures are not as reliable as at the blastocyst stage.

This study should be considered in follow-up to a recently reported prospective study from PIVET which examined 1213 IVF-naïve women proceeding directly into 1761 IVF±ICSI treatments [33]. With a view to countering poor prognosis factors, 21.5% of the women utilized rGH with the aim of generating optimal embryology and clinical outcomes. Indeed, the findings showed significant improvement in embryo utilization rates for older women who had additional poor prognosis factors, namely incremental cycles ≥ 3 or failure to achieve cryopreserved embryos. However, these benefits failed to translate into an improved pregnancy or live birth productivity rate nor a reduction in miscarriage rates. However, the study was limited by the low numbers of women who had several poor-prognosis factors. Nonetheless, a note of caution emerged from the study as younger women who did not receive rGH had significantly better live birth outcomes, regardless of the number of poor-prognosis factors identified. It was concluded that future adjuvant studies should be focused on older women. Hence this much larger retrospective study concludes very similar findings to the prospective study.

Much to our disappointment, this retrospective study, undertaken on a much larger population of women than all the earlier encouraging studies, showed essentially no clinical benefit from either rGH or melatonin. The global data showed that younger women who did not utilise adjuvants, had a significantly higher live birth productivity rate per initiated cycle. Concerning the embryo utilization rates, again the result was similarly disappointing as no clear improvement could be shown from either the melatonin or rGH adjuvants, albeit that older woman (in the group 40-44 years) showed a marginally better rate with rGH. However, with respect to those women who had poor embryo quality (nil embryos cryopreserved), there was indeed a significant improvement in embryo utilization rates from rGH, but not from melatonin. The rGH benefit was seen across all the age groups for those women categorised with either high or low ovarian reserve but who had high oocyte numbers retrieved. In fact, the benefit was most marked among the younger groups of women. These findings on improved embryo utilization rates for women with poor embryo quality is mirrored in other recent reports elsewhere [15, 34-38] and concurs with physiological studies showing improved oocyte competence at many levels [39-42]. However, our study involves a large cohort and controls for many variables in an historic, disciplined clinical practice where blastocyst culture and single embryo transfers are the standard.

With respect to melatonin, no improvements were detected, neither in oocyte numbers, nor embryo utilization rates nor live birth rates. Despite the potential physiological benefits, our study matches the first encouraging report which also failed to demonstrate any clinical benefits from melatonin as an adjuvant in IVF [30].

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 can improve embryo utilization rates in women who generate embryos of poor quality, there is no detectable improvement in live birth rates. Melatonin had no influence on any embryology nor clinical outcomes.

Compliance with ethical standards

Acknowledgments

We are grateful for the close working relationship between PIVET® Medical Centre and CLINIPATH® Pathology which carried out the assays involved in the IGF profile and is also accredited by NATA. The nursing team at PIVET have been fastidious in detailing clinical outcomes into registers, thereafter into the Filemaker database. Nurse Alison Pusey has been especially successful in tracking the outcome of each pregnancy, many of which resulted in deliveries in regional locations and sometimes overseas.

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.

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).

References

- [1] Yovich JL, Craft IL. Founding pioneers of IVF: Independent innovative researchers generating livebirths within 4 years of the first birth. *Reprod Biol.* 2018; 18: 317-323.
- [2] Yovich JL. Founding pioneers of IVF Update: Independent innovative researchers generating livebirths within 4 years of the first birth. *Reprod Biol.* 2020; 20: 111-113.
- [3] Yovich JL, Zaidi S, Nguyen MDK, Hinchliffe PM. Measuring IGF-1 and IGFBP-3 profiles in women seeking assisted reproduction; relationship to ovarian reserve parameters (Study 2). *GSC Biol & Pharmaceutical Sciences.* 2020; 13(02): 035-053.

- [4] Keane K, Cruzat VF, Wagle S, Chaudhary N, Newsholme P, Yovich J. Specific ranges of anti-Mullerian hormone and antral follicle count correlate to provide a prognostic indicator for IVF outcome. *Reprod Biol.* 2017; 17: 51-59.
- [5] Yovich JL, Zaidi S, Nguyen MDK, Hinchliffe PM. Measuring IGF-1 and IGFBP-3 profiles in women seeking assisted reproduction; relationship to serum growth hormone levels (Study 3). *GSC Biol & Pharmaceutical Sciences.* 2020; 13(03): 032-053.
- [6] Yovich JL, Zaidi S, Nguyen MDK, Hinchliffe PM. Measuring IGF-1 and IGFBP-3 profiles in women seeking assisted reproduction; relationship to clinical parameters (Study 1). *J Pers Med.* 2020; 10(3): 122, 1-15
- [7] Yovich JL, Conceicao JL, Wong J, Marjanovic N, Wicks R, Hinchliffe PM. Fertilization by ICSI generates a higher number of live births than IVF in a pioneer facility applying >90% single blastocyst-stage embryo transfers. *GSC Biol & Pharmaceutical Sciences.* 2021; 15(01): 087-103.
- [8] Mariappen U, Keane KN, Hinchliffe PM, Dhaliwal SS, Yovich JL. Neither male age nor semen parameters influence clinical pregnancy or live birth outcomes from IVF. *Reprod Biol.* 2018; 18: 324-329.
- [9] Yovich JL, Zaidi S, Nguyen MDK, Hinchliffe PM. Measuring IGF-1 and IGFBP-3 profiles in women seeking assisted reproduction; relevance to clinical outcomes from in vitro fertilization (Study 5). *GSC Biol & Pharmaceutical Sciences.* 2020; 13(03): 079-096.
- [10] Yovich JL, Zaidi S, Nguyen MDK, Hinchliffe PM. Measuring IGF-1 and IGFBP-3 profiles in women seeking assisted reproduction; response of women categorised as poor prognosis to growth hormone adjuvant therapy (Study 4). *GSC Biol & Pharmaceutical Sciences.* 2020; 13(03): 064-078.
- [11] Keane KN, Ye Y, Regan SLP, Dhaliwal SS, Yovich JL. Live birth outcomes of vitrified embryos generated under growth hormone stimulation are improved for women categorized as poor-prognosis. *Clin Exp Reprod Med.* 2019; 46(40): 178-188.
- [12] Yovich JL, Ye Y, Regan SLP, Keane KN. The evolving concept of poor-prognosis for women undertaking IVF and the notion of growth hormone as an adjuvant; a single-center viewpoint. *Front Endocrinol.* 2019; 10(808): 1-14.
- [13] Keane KN, Hinchliffe PM, Namdar N, Conceicao JL, Newsholme P, Yovich JL. Novel dehydroepiandrosterone troche supplementation improves the serum androgen profile of women undergoing in vitro fertilization. *Drug Des Devel Ther.* 2015; 9: 5569–78.
- [14] Keane KN, Hinchliffe PM, Rowlands PK, Borude G, Srinivasan S, Dhaliwal SS, Yovich JL. DHEA Supplementation confers no additional benefit to that of growth hormone on pregnancy and live birth rates in IVF patients categorized as poor prognosis. *Front Endocrinol.* 2018; 9(14): 1-11.
- [15] Tesarik J, Galán-Lázaro M, Conde-López C, Chiara-Rapisarda AM, Mendoza-Tesarik R. The effect of GH administration on oocyte and zygote quality in young women with repeated implantation failure after IVF. *Front Endocrinol.* 2020; 11: 519-572, 1-7.
- [16] Yovich JL, Regan SL, Zaidi SN, Keane KN. The concept of growth hormone deficiency affecting clinical prognosis in IVF. *Front Endocrinol.* 2019; 10(650): 1-9.
- [17] Yovich JL, Ye Y, Keane KN. Growth hormone adjuvant trial for poor responders undergoing IVF. *Eur J Obstet Gynecol.* 2019; 236: 249-251.
- [18] Bortoletto P, Spandorfer S. Growth hormone: in search of the holy grail for poor responders (or a felony). *Fertil Steril.* 2020; 114(1): 63-64.
- [19] Newman JE, Paul RC, Chambers GM. Assisted reproductive technology in Australia and New Zealand 2018. Sydney: National Perinatal Epidemiology and Statistics Unit, the University of New South Wales, Sydney. 2020; 1-83.
- [20] Your IVF Success Estimator. Find an Australian IVF clinic. Australian & New Zealand Assisted Reproduction Database. University of New South Wales. 2020 [cited 3 March 2021]. Available from <https://www.yourivfsuccess.com.au>
- [21] Yovich JL. How to Prepare the Egg and Embryo to Maximise IVF Success. In: *Monitoring the stimulated IVF cycle. Section II: Stimulation for IVF* (Eds: Gabor T Kovacs, Anthony J Rutherford, David K Gardner). Cambridge University Press, Cambridge. 2019; 94-120.
- [22] Yovich J, Stanger J, Hinchliffe P. Targeted gonadotrophin stimulation using the PIVET algorithm markedly reduces the risk of OHSS. *Reprod Biomed Online.* 2012; 24(3): 281-292.

- [23] Yovich JL, Alsbjerg B, Conceicao JL, Hinchliffe PM, Keane KN. PIVET rFSH dosing algorithms for individualized controlled ovarian stimulation enables optimized pregnancy productivity rates and avoidance of ovarian hyperstimulation syndrome. *Drug Des Devel Ther.* 2016; 10: 2561–2573.
- [24] Yovich JL, Hinchliffe PM, Lingam S, Srinivasan S, Keane KN. Adjusting the PIVET rFSH dosing algorithm for the biosimilar Bemfola product. *J Fertil In vitro IVF Worldw Reprod Med Genet Stem Cell Biol*, 2018, 5(3), 1-4
- [25] Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. *Reprod Biomed Online*, 2005, 11, 300-308
- [26] Yovich JL, Conceicao JL, Marjanovich N, Ye Y, Hinchliffe PM, Dhaliwal SS, Keane KN. An ICSI rate of 90% minimizes complete failed fertilization and provides satisfactory implantation rates without elevating fetal abnormalities. *Reprod Biol*, 2018, 18, 301-311
- [27] Mustafa KB, Keane KN, Walz NL, Mitrovic KI, Hinchliffe PM, Yovich JL. Live Birth Rates are satisfactory following multiple IVF treatment cycles in poor prognosis patients. *Reprod Biol*, 2017, 17, 34-41
- [28] Yovich JL, Conceicao J, Hinchliffe P, Keane K. Which blastocysts should be considered for genetic screening? *Hum Reprod*, 2015, 30, 1743-1745
- [29] Farquhar C. Add-ons for assisted reproductive technology: can we be honest here? *Fertil Steril*, 2019, 112(6), 971-972
- [30] Fernando S, Osianlis T, Vollenhoven B, Wallace E, Rombauts L. A pilot double-blind randomised placebo-controlled dose-response trial assessing the effects of melatonin on infertility treatment (MIART): study protocol. *BMJ Open.* 2014; 4(8): e0059866, 1-8.
- [31] Yovich JL, Stanger JD, Keane KN. Cumulative live birth rate: An outmoded term. *JFIV Reprod Med Genet.* 2016; 4: 165
- [32] Hammer ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for education and data analysis. *Palaentologia Electronica.* 2001; 4(1): 1-9.
- [33] Yovich JL, Srinivasan S, Sillender M, Gaur S, Rowlands P, Hinchliffe PM. Using growth hormone as an adjuvant in IVF: Live birth outcomes from various poor prognosis scenarios. *GSC Biol & Pharmaceutical Sciences.* 2021; 15(01): 063-080.
- [34] Norman RJ, Alvino H, Hull LM, Mol BW, Hart RJ, Kelly T-L, et al. Human growth hormone for poor responders: a randomized placebo-controlled trial provides no evidence for improved live birth rate. *Reprod Biomed Online.* 2019; 38: 908–15.
- [35] Li J, Chen Q, Wang J, Huang G, Ye H. Does growth hormone supplementation improve oocyte competence and IVF outcomes in patients with poor embryonic development? A randomized controlled trial. *BMC Pregnancy and Childbirth.* 2020; 20(310): 1-10.
- [36] Zhang Y, Zhang C, Shu J, Guo J, Chang H-M, Leung PCK, Sheng J-Z, Huang H. Adjuvant treatment strategies in ovarian stimulation for poor responders undergoing IVF: a systematic review and network meta-analysis. *Hum Reprod Update.* 2020; 26(2): 247-263.
- [37] Yang P, Wu R, Zhang H. The effect of growth hormone supplementation in poor ovarian responders undergoing IVF or ICSI: a meta-analysis of randomized controlled trials. *Reprod Biol Endocrinol.* 2020; 18(1): 76, 1-10.
- [38] Tesarik J, Yovich JL, Menezo Y. Editorial: Growth hormone in Fertility and infertility: physiology, pathology, diagnosis and treatment. *Front Endocrinol.* 2021; 12: 621722.
- [39] Devesa J, Caicedo D. The role of growth hormone on ovarian functioning and ovarian angiogenesis. *Front Endocrinol.* 2019; 10: 1-16.
- [40] Ipsa E, Cruzat VF, Kagize JN, Yovich JL, Keane KN. Growth Hormone and Insulin-like growth factor in reproductive tissues. *Front Endocrinol.* 2019; 777: 1-14.
- [41] Weall BM, Al-Samerria S, Conceicao J, Yovich JL, Almahbobi G. A direct action for GH in improvement of oocyte quality in poor-responder patients. *Reproduction.* 2015; 149: 147-154.
- [42] Regan SLP, Knight PG, Yovich JL, Arfuso F, Dharmarajan A. Growth hormone during in vitro fertilization in older women modulates the density of receptors in granulosa cells, with improved pregnancy outcomes. *Fertil Steril.* 2018; 110: 1298–131.