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Abstract: Objective: To determine the influence of growth hormone (GH) on clinical outcomes in IVF patients. Design: Single-centre observational study, where IVF cycles from women prescribed adjuvants, were compared to cycles where they did not receive adjuvant. Setting: Private IVF facility with university affiliation. Patients: Poor-prognosis patients with advanced maternal age, low ovarian reserve, low quality embryos and offered adjuvant therapy. Intervention: None. Main Outcome Measures: Differences in clinical pregnancy and live birth chance and rates. Results: 371 IVF patients with 509 IVF cycles were analysed, and comprised 286 cycles where no adjuvant was used, and 223 cycles where GH only was used. Clinical pregnancy and live birth rates were significantly greater with GH, despite patients being significantly older with lower ovarian reserve. Patient age, quality of transferred embryo and GH supplementation were the only significant independent predictors of clinical pregnancy (Odds-Ratio: 0.89, 2.52 and 2.22, $p<0.002$, respectively) and live birth chance (Odds-Ratio: 0.88, 2.43 and 3.95, $p<0.008$, respectively). Following adjustment for patient age and transferred embryo quality, GH increased clinical pregnancy chance by 2.50-fold (95% CI: 1.04 - 6.00, $p<0.041$) and live birth chance by 5.89-fold (95% CI: 1.92 - 18.08, $p<0.002$). Conclusion: These data provided further evidence to indicate that GH may support more live births, particularly in younger women, and is the first GH-IVF study to simultaneously incorporate AFC, AMH and embryo quality assessment. It also appears that embryos generated under GH have a better implantation potential, but whether the biological mechanism is embryo- or endometrium-mediated is unclear.

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28 April 2017

Dear Antonio,

Please receive the attached manuscript entitled:

Growth Hormone Supplementation in IVF Cycles Improves Pregnancy and Live Birth Rates in Poor-Prognosis Patients up to Age 42 Years

This manuscript arises from collaborative work between PIVET Medical Centre and Curtin University. The authorship line-up is:

Kevin N Keane, John L Yovich*, Anahita Hamidi, Peter M Hinchliffe & Satvinder S Dhaliwal.

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Please consider this case-controlled study using Growth Hormone as an adjuvant for women classified as poor-prognosis based on 4 criteria.

We have been researching Growth Hormone adjuvant therapy in IVF for 15 years, publishing an earlier report from a 5-year study 2002-2006 (Yovich & Stanger; 2010, RBM Online). This study embraces an ensuing timeframe of 7 years 9 months (2008-2015) and tracks all treatment cycles through to pregnancy with outcomes including live-births as the main parameter. It is a clear data set, where no other adjuvants were used. Applying logistic regression analysis for numerous variables we defined clear benefits for women up to, but not beyond, age 42 years with the highest livebirths in the 35-39 year age-range (OR indicates 6-fold improvement).

There are no conflicts of interest to report here; in particular no pharmaceutical grants were involved. Thank you for considering our manuscript for Fertility and Sterility.

Yours sincerely,

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Growth Hormone Supplementation in IVF Cycles Improves Pregnancy and Live Birth Rates in Poor-Prognosis Patients up to Age 42 Years

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Short Title: Growth Hormone in Poor-Prognosis IVF Cases.

Key Words: Growth Hormone; In Vitro Fertilisation; Embryo Quality, Adjuvants.

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Abstract

Objective: To determine the influence of growth hormone (GH) on clinical outcomes in IVF patients. **Design:** Single-centre observational study, where IVF cycles from women prescribed adjuvants, were compared to cycles where they did not receive adjuvant. **Setting:** Private IVF facility with university affiliation. **Patients:** Poor-prognosis patients with advanced maternal age, low ovarian reserve, low quality embryos and offered adjuvant therapy. **Intervention:** None. **Main Outcome Measures:** Differences in clinical pregnancy and live birth chance and rates. **Results:** 371 IVF patients with 509 IVF cycles were analysed, and comprised 286 cycles where no adjuvant was used, and 223 cycles where GH only was used. Clinical pregnancy and live birth rates were significantly greater with GH, despite patients being significantly older with lower ovarian reserve. Patient age, quality of transferred embryo and GH supplementation were the only significant independent predictors of clinical pregnancy (Odds-Ratio: 0.89, 2.52 and 2.22, $p<0.002$, respectively) and live birth chance (Odds-Ratio: 0.88, 2.43 and 3.95, $p<0.008$, respectively). Following adjustment for patient age and transferred embryo quality, GH increased clinical pregnancy chance by 2.50-fold (95% CI: 1.04 – 6.00, $p<0.041$) and live birth chance by 5.89-fold (95% CI: 1.92 – 18.08, $p<0.002$). **Conclusion:** These data provided further evidence to indicate that GH may support more live births, particularly in younger women, and is the first GH-IVF study to simultaneously incorporate AFC, AMH and embryo quality assessment. It also appears that embryos generated under GH have a better implantation potential, but whether the biological mechanism is embryo- or endometrium-mediated is unclear.

Introduction

Many international *In Vitro* Fertilisation (IVF) clinics supplement patients with various adjuvant therapies in order to enhance IVF success rates, particularly for those women who are categorised as "poor prognosis" according to the Bologna criteria [1]. Some of the most common adjuvant therapies include steroid supplementation, such as dehydroepiandrosterone (DHEA) or oestradiol, immune therapy including intravenous immunoglobulin administration, and growth hormone (GH) supplementation [2]. Yet the true beneficial effects of these therapies are hotly debated [2]. This is the result of various

studies that have demonstrated either inconsistent or opposite findings, utilised small patient cohorts, or conducted poorly designed trials that were not blinded or were not placebo controlled. However, in the context of IVF, strict double blind, placebo-controlled, randomised clinical trials (RCT's) are difficult to complete, as has been observed with the recent early closure of the LIGHT study (Livebirth rate *In vitro* fertilisation and Growth Hormone Treatment), in Australia and New Zealand [3]. Fully blinded RCT's in IVF are problematic mainly because of patient recruitment issues, where ageing women prefer not to commit several months of their reproductive lifespan to a placebo agent that ultimately may not help them attain pregnancy. Instead, eager patients tend to opt for any additional treatment, cost permitting, that would potentially help them to fall pregnant. Consequently, while retrospective or observational studies are not "optimally" designed, they still provide important information concerning therapeutic interventions in IVF, especially where sufficiently powered RCT's are lacking, as observed with GH studies.

Since 1988, several trials including observational, sequential crossover and RCT's have been performed to evaluate the clinical benefit of GH supplementation in IVF patients [4]. The first report by Jacob's group in 1988 showed that GH improved ovarian sensitivity to human menopausal gonadotrophins in women with hypogonadotrophic-hypogonadism [5]. Subsequently, several small double blind placebo-controlled RCT's were initiated, but failed to reveal improvements in ovarian response or clinical parameters, including number of oocytes retrieved and fertilised [6-8]. In addition, while Busacca et al. (1996) found that GH decreased duration of ovarian stimulation, along with reducing FSH dose and concomitantly increasing the number of developing follicles [9], other groups such as Levy et al. (1993) and Suikkari et al. (1996), observed no such significant change [10, 11]. However, since the mid 2000's, interest in GH as adjuvant therapy in IVF treatment has been resurrected by several interesting reports.

The study by Tesarik et al. (2005), showed that GH reduced the number of miscarriages in patients with advanced maternal age (over 40 years), and thus increased the live birth delivery rate [12]. Interestingly, GH had little effect on pregnancy rates or number of oocytes retrieved in these patients. Similarly, an earlier study from our clinic demonstrated that GH improved live birth rates (20% v 7%) and reduced miscarriage

rates (35 % v 48%) in a sequential crossover study [13]. Increased pregnancy rates for fresh and frozen cycles was also observed [13]. Conversely, others have shown that although GH can increase oocyte and embryo retrievals, it failed to improve pregnancy rates in poor responders, which further adds complexity to the potential benefits of GH [14]. More recently, in a prospective cohort with a concerted effort to reduce the cost associated with GH therapy in IVF, it was found that low dose GH (0.5 I.U. per day) increased the clinical pregnancy rate in poor responders, while also improving the number of top quality embryos produced [15]. Taken together, these data indicated that any positive effect from GH, may center on improved embryo and oocyte quality, which may lead to reduced aneuploidy and subsequent miscarriages in poor prognosis or older patients. In the current report, using a new cohort of patients, we add further weight to the hypothesis that GH improves IVF success rates by reducing miscarriage rates, thereby increasing the delivery rate. Importantly, the study also shows that GH did not significantly affect the number of low or high embryos retrieved, which confounds the potential mechanism by which this adjuvant may exert its benefits.

Materials and Methods

Study Period and Participants

This retrospective study covered 2202 women who had 3505 initiated IVF cycles from 1 April 2008 to 31 December 2015, 3427 of which proceeded to ovum pick-up (OPU) by TVOA (transvaginal oocyte aspiration). The current study focused on a subset of these IVF patients who were offered IVF adjuvants because they were classified as poor-prognosis cases on the basis of one or more of the following criteria: (i) women with fewer than 4 metaphase II (M II) oocytes although receiving maximal FSH stimulation (i.e. 450 IU/day); (ii) women with embryos where the majority of embryos (>55%) showed marked fragmentation and were graded poor quality rating ≤1.5 (out of a possible 4.0 points) in our long-standing embryo-grading system [16]; (iii) women with repetitive fresh or frozen embryo transfers (≥3 transfers) without pregnancy and where diminished egg or embryo quality was identified by the laboratory; (iv) women aged ≥40 years who had at least 1 failed IVF cycle.

These patients selected GH on the basis of several factors, one of which was cost (since patients were required to pay). Some women chose to undergo a single cycle without GH treatment, progressing to GH next time if not conceiving. However, once GH was selected, patients accepted the sequential crossover study design whereby a course of GH could not be repeated within 6 months. They could choose to utilise no adjuvants, DHEA or melatonin on repeat IVF cycles after a failed GH adjuvant cycle. Consequently, women "qualified" for study inclusion if they met the criteria above and had been offered GH, DHEA or melatonin at any point within the defined timeframe of the study period. However, only cycles within the study period where no adjuvant (-)GH, or GH alone (+)GH was utilised, were subject to analysis. Therefore, the dataset did not include any data from any initiated IVF cycle outside the study date range, and only includes cycles from "qualifying" women, and consisted of cycles (+)GH or (-)GH (no adjuvant therapy), and excluded cycles with DHEA or melatonin.

Overall, 484 women (22.0% of total group) were offered some form of adjuvant treatment (GH, DHEA or melatonin) during the study period, and the initial analyses were conducted on their corresponding 1488 fresh IVF cycles (42.5% of total cycles). After removal of irrelevant cycles with no fresh transfer, 1048 IVF treatments remained where fresh embryo transfer (ET) occurred (Figure 1). However, only those cycles where no adjuvant therapy [(-)GH, n=286] or GH only [(+)GH, n=223] was administered are the subject of analysis in this article.

Clinical Management

GH in the form of Scitropin or Saizen was administered during the preceding menstrual cycle. All patients were stimulated with recombinant FSH using specific dosage algorithms as defined recently [17], and in most cases (44.8% of cycles) using an antagonist protocol. Other older patients received a flare-agonist regimen (34.4%) or specialised down regulation protocols (20.8%) [18] (Table 1). Ovulation was triggered with human chorionic gonadotrophin (HCG). TVOA was undertaken 36 h post trigger under IV sedation using a PIVET-Cook double-lumen flushing/aspiration needle (Cook, Australia). The luteal phase was managed using HCG support [19]. Additional support hormones were given as required (oestradiol, progesterone or combined

oestradiol/progesterone pessary). Where ≥12 oocytes were recovered, progesterone pessaries replaced HCG injections.

Embryo culture and assessment

Oocytes were cultured for 4–5 h post collection before insemination with motile spermatozoa (100,000/ml) for IVF, or denuded with hyaluronidase and mature oocytes injected using ICSI. Day-3 embryos were graded using a four-point system, with half points increments (grade 4 = 8+ cells no fragmentation and early compaction evident; grade 3 = 7–9 cells, no fragmentation and no compaction; grade 2 = slow cleavage and/or >20% fragmentation; grade 1 = arrested or significantly fragmented embryos). Embryos graded ≤1.5 were discarded, those graded 2.0 were defined as "low quality" and those between 2.5 and 4.0 were deemed "high quality". Day-5 embryos were graded using the Gardner scoring system for blastocysts [20].

Embryos were transferred to the uterus in 10–20 μl of culture media using the Cook double-catheter system (K-JITS-2005; Cook). They were deposited just short of the fundus with a clear flash identified on ultrasound and a negative check on the transfer catheter. Although the clinic has a strong policy of single embryo transfer, cases categorised as poor prognosis can receive up to two Day-3 embryos (in 103 cycles (-)GH and 134 (+)GH), or on a rare occasion, three Day-3 embryos (in 2 cycles (-)GH and 1 cycle (+)GH). Single blastocysts were transferred in a minority of cycles.

Data Analysis and Statistics

The main outcomes of this study were chance and rate of clinical pregnancies and live births. Logistic regression was used to assess the independent contributions of individual confounding parameters on these outcomes such as age, body mass index (BMI), anti-mullerian hormone (AMH) level, antral follicle count (AFC), stimulation protocol type, quality, developmental stage and number of embryos transferred, in addition to the number of patient infertility factors and previous IVF attempts. The unadjusted effect of GH administration on these binary outcomes was also assessed. The effect of each variable was expressed as an odds ratio (OR) with associated 95% confidence interval (CI). Stepwise multiple logistic regression analyses enabled the

determination of the minimum number of independent variables that could be used for predicting pregnancy and/or live birth chance. The coefficients of the independent variables from each of the final logistic regression models were used to calculate OR and CI of pregnancy and/or live birth chance due to the presence or absence of GH. Continuous variables for the (-)GH and (+)GH groups were compared using two-sample t-tests and categorical variables were compared using Fisher's Exact Chi-Squared tests.

Patient Consent and Ethical Approval

Our clinic is accredited with the Reproductive Technology Accreditation Committee, and the Reproductive Technology Council of Western Australia. These agencies monitor all activities. Specific ethics approval was not required for this study as all procedures and blood tests were embraced by routine approved clinical protocols. However, reporting of the data was approved under Curtin University Ethics Committee approval no. RD_25-10 general approval for retrospective data analysis 2015. In addition, as part of our documentation system, written-, informed-consent was obtained from each participant that accepted the use of adjuvants, and they were required to pay for these adjuvants over and above the IVF treatment charges.

Results

Overview of Patient Demographics according to Pregnancy and Live Birth Success

The majority of the cycles analysed in this poor-prognosis cohort resulted in no clinical pregnancy (85.1%). However, the overall pregnancy rate for this group was 14.9%, while the live birth rate was 10.8% (miscarriage rate of 27.6%, 21/76) (Table 1). The majority of women were aged between 35 and 44 years (78.0%), with an AFC of 5-8 follicles (36.8%), and most received antagonist stimulation (44.8%). For those that became pregnant, they tended to be younger (mean age of 36.3 to 37.4 versus 38.9 years), had more embryos cryopreserved (mean of 0.9 to 1.0 versus 0.7 embryos), and had a higher proportion of high quality embryos at OPU (mean of 43.0 to 43.9 versus 34.6%) (Table 1). The cohort that went on to have a successful live birth were significantly younger (mean age of 36.3 years), and also had a significantly greater proportion of high quality embryos at OPU in comparison to those who did not become pregnant (43.9%

versus 34.6%) (Table 1). There was no significant difference in the mean number of embryos transferred, fertilisation rate, mean oocytes retrieved or oocyte/embryo utilisation rates among those that failed to become pregnant, those that did become pregnant, those that miscarried and those that had a live birth (Table 1).

No adjuvant (-)GH, was administered in 56.2% of analysed cycles, while (+)GH was used in the remainder (43.8% of cycles). However, in all of the cycles where there was a live birth, 72.7% of live birth were derived from a (+)GH cycle, while only 27.3% of cycles with live births came from (-)GH cycles (Table 1). Furthermore, in all cycles were a miscarriage occurred, 28.6% were derived from a (+)GH cycle, while the majority (71.4%) were from (-)GH cycles (Table 1). Overall, the pregnancy rate with (+)GH was 20.6 % (46/223) versus 10.5% (30/286) for (-)GH, and the live birth rate (+)GH was 17.9 % (40/223) versus 5.2% (15/286) for (-)GH (Table 2).

Overview of (-)GH and (+)GH Cycle Groups

From the included patient cohort, there was no significant difference between (+)GH cycles and (-)GH cycles with regard to the mean BMI, mean oocytes retrieved, mean two pronuclei generated, fertilisation rate, and proportion of high, medium or low quality embryos generated after OPU (Table 2). However, the (+)GH cohort was significantly older (39.4 versus 37.9 years, p=0.001), had a lower mean AMH (6.2 versus 10.9 pmol/L, p=0.004), but had higher oocyte (p=0.001) and embryo (p=0.001) utilization rates (Table 2).

Univariate and Multivariate Analysis using Logistic Regression

Table 3 presents calculated clinical pregnancy and live birth odd ratios for each individual variable. Only patient age, transferred embryo development stage (blastocyst versus cleavage stage), transferred embryo quality, and the presence of (+)GH were significant predictors of clinical pregnancy and/or live birth chance. Patient AMH, AFC, BMI, number of embryos transferred, stimulation protocol type, infertility factors or previous IVF attempts did not influence clinical pregnancy and/or live birth chance significantly (Table 3). When stepwise multiple logistic regression was performed using all terms, only patient age, transferred embryo quality, and presence of (+)GH were

retained and were all significant. Increasing patient age decreased the chance of clinical pregnancy and/or live birth by about 11% per advancing year. When adjusted for patient age and presence or absence of (+)GH, the chance of clinical pregnancy was increased by 3.9- and 13.2-fold when high quality day-3 or high quality blastocysts were transferred, respectively ($p<0.002$) (Table 3). This increased chance was significant and similar for live birth outcomes (3.1 and 9.5-fold, respectively) ($p<0.008$). Most importantly, following adjustment for patient age and transferred embryo quality, (+)GH significantly increased the chance of clinical pregnancy success by 2.5-fold (95%C.I. 1.04-6.00, $p=0.041$) and significantly increased the chance of live birth success by 5.9-fold (95%C.I. 1.92-18.1, $p=0.002$) (Table 3).

Interaction of Patient Age and (+)GH Treatment

When the data was analysed according to age groups, the effect of (+)GH was dependent on patient age. Those who were less than 39 years were at least three times more likely to achieve a pregnancy in (+)GH cycles (Table 4). However, (+)GH did not appear to alter the likelihood of successful pregnancy in those aged 40 and above (Table 4). A similar response was also observed for chance of live birth, with those less than 35 years, or between 35 and 39 inclusive, being 5.2- and 9.5-times more likely to achieve a live birth in (+)GH cycles, respectively (Table 4). There was a trend towards a positive effect of (+)GH on live birth outcomes (OR: 2.7, 95% CI 0.8 – 8.8) in those aged 40 – 44, but this was not significant ($p=0.095$). No pregnancies or live births were achieved in women 45 years and older in this cohort (Table 4). However, sub-group analysis revealed that although (+)GH did not significantly affect the chance of successful pregnancy in 40 and 41 year olds (OR: 1.6, 95% CI 0.5 – 4.9, $p=0.425$), (+)GH slightly but significantly increased the chance of live birth in this group by 5-fold (OR: 5.1, 95% CI 1.0 – 25.5, $p=0.045$) (Table 4).

Interaction of Transferred Embryo Quality and (+)GH Treatment

When the data was analysed according to the morphological quality of the transferred embryo, the effect of (+)GH was dependent on this variable. The majority of cycles (88.0%) included the transfer of a Day-3 cleavage stage embryo, while only 61

cycles (12.0%) involved the transfer of a Day-5 blastocyst (Table 5). Consequently, we focused on the interaction between transferred Day-3 cleavage stage embryo quality and (+)GH (Table 5). High quality Day-3 embryos with 8+ cells, no fragmentation and an early compaction evident, led to greater pregnancy chance and greater live birth chance in comparison to low quality Day-3 embryos with slow cleavage and/or >20% fragmentation (Table 5). (+)GH did not influence pregnancy probability when high quality embryos were transferred, but significantly enhanced live birth chance by 3.1-fold (95% CI 1.4 – 7.0, p=0.005) when these high quality embryos were transferred (Table 5). Conversely, (+)GH increased pregnancy chance by 3.6-fold (95% CI 1.3 – 10.4, p=0.017) when low quality embryos were transferred, but the increase in live birth chance was not significant (3.1, 0.95 – 10.2, p=0.062) (Table 5).

Discussion

In the current observational study, we showed that patient age, the quality of transferred embryos and the utilisation of growth hormone (GH), were significant predictors of clinical pregnancy and live births in IVF patients categorised as poor-prognosis, with advancing maternal age, low ovarian reserve makers, previous IVF failure or previous poor quality embryos. Other patient characteristics including BMI, AMH, AFC, number of infertility factors or previous IVF attempts did not have an independent effect on clinical pregnancy or live birth chance in this cohort. Specifically, we have demonstrated that (+)GH increased the chance of these outcomes in women aged less than 40 years old. Furthermore, significantly more live births were observed in the (+)GH group who had an older average age (mean difference of 1.5 years; 37.9 (-)GH versus 39.4 years (+)GH), and a lower average serum AMH value, and consequently could be viewed as a very poor-prognosis group. However, sub-analyses also demonstrated a slight but significant live birth benefit in patients who were aged 40 and 41 years, but no effect was observed for pregnancy chance here. Taken together, these data illustrated a clear age-dependent effect from GH supplementation, which appeared to have more positive results in younger poor-prognosis IVF patients. These findings further intensify the debate regarding the potential advantageous effects of GH adjuvant

treatment in assisted reproductive technologies, particularly in relation to enhanced live birth rates, the ultimate outcome of IVF success.

These results also echo our earlier work [13], and comparable data derived from an RCT by Tesarik et al. (2005) [12], which indicated that GH may reduce aneuploidies, leading to lower miscarriage and higher live births. However, our current dataset contrasts with this RCT study where patients had a mean age of 42 years, in that clinical pregnancies and live birth rates were not affected by (+)GH in our older patient group (above 41 years). This disparity may be due to the difference in the number of transferred embryos in the studies, where on average Tesarik et al. (2005) transferred 3.5 and 4.2 embryos (-)GH and (+)GH, respectively, and we transferred 1.37 and 1.61 embryos, respectively. However, number of embryos transferred did not alter clinical pregnancy or live birth chance independently in our study. In terms of oocyte and embryo utilisation rates, these were also elevated (+)GH, but there was no difference in the mean number of oocytes retrieved at OPU, or oocytes with two pronuclei generated. Other reports showed that (+)GH increased oocyte and embryo retrieval [14], and generated more oocytes with two pronuclei [21]. However, pregnancy and live birth rates were not altered significantly in these studies [14, 21].

Poor prognosis patients defined by the Bologna Criteria have at least two of three clinical parameters which include, advanced maternal age (>39 years), a poor ovarian response with 3 or less oocytes collected in a previous cycle, or an abnormal ovarian reserve compromising of low antral follicle count (AFC) (< 7 follicles), or low AMH (< 8 pmol/L) [1]. Most patients (33 – 41%) in each group had between 5-8 follicles and were graded as AFC category D using our clinical criteria [17, 22], and the (+)GH group had a significantly reduced serum AMH level and were older on average. In spite of this perceived very poor ovarian reserve and advance maternal age, we are the first to report that (+)GH improved oocyte and embryo utilisation rates, live births and miscarriage rates in patients with reduced AMH and similarly low AFC ratings [23]. We also investigated the effect of (+)GH on patients with different AFC grading, but neither AFC or the presence or absence of GH significantly altered clinical pregnancy or live birth chance in different AFC groupings. However, since patient ovarian reserve has not been

described in any IVF study utilising GH [23], direct comparison of our AFC findings with other studies is restricted.

Almost half of the patients in our cohort were stimulated using an antagonist protocol (44.8%). However, stimulation type did not independently modulate the chance of clinical pregnancy or live birth. Furthermore, when adjusting for stimulation protocol, (+)GH increased clinical pregnancy chance by 2.2-fold (p=0.002), and live births by 3.9-fold (p=0.000), but again protocol type had no impact. Interestingly, other reports have suggested that GH significantly increases the number of embryo transferred in flare agonist cycles [12, 21], but this was observed across all stimulation protocols in our study (1.38 versus 1.60 embryos transferred (-)GH & (+)GH, respectively). However, the number of embryos transferred did not significantly or independently affect clinical outcomes.

As previously reported [24], the quality of the transferred embryo was shown to be a key player in successful pregnancy or live births, and it was confirmed in the current study. Due to the poor-prognosis nature of the patients, the majority of embryos transferred (88%) were Day-3 cleavage stage embryos. Only 12% of transfers utilised blastocyst culture, and consequently, analysis of blastocyst transfer was limited. Nonetheless, the highest pregnancy and live birth rates were observed when high quality blastocysts or high quality Day-3 embryos were transferred, and these had an independent effect on clinical outcomes. Interestingly, when adjusting for transferred embryo quality, (+)GH increased clinical pregnancy chance significantly, and there was a trend towards increased live birth chance when low quality Day-3 embryos with slow cleavage and/or >20% fragmentation were transferred. Conversely, live birth chances were markedly significantly when high quality Day-3 embryos with no fragmentation were transferred. It appears that embryos generated under GH supplementation may have a better implantation potential but whether the mechanism is embryo- or endometrium-related is unclear from this study.

The authors speculated that GH supplementation might lead to more usable oocytes and embryos, and thus this inferred that GH had an impact on egg quality, which has been suggested previously [12, 13]. However, when embryo quality was determined using morphological analysis, it was found that GH did not alter the quantity of embryos

in low, medium or high quality embryo categories at OPU. Conversely, it was recently shown that low-dose GH was able to slightly but significantly enhance the number of top graded embryos in poor-prognosis patients (p=0.04) [15]. Therefore, it is surprising that this was not demonstrated in the current study. It may be the case that GH supplementation improves embryo quality that cannot be detected through morphological examination, or GH may improve endometrium receptivity as has been previously suggested in animal studies [25]. However, this has not yet been explored in humans.

In conclusion, this new observational GH study, the first to include aspects of analysis such as AFC, AMH, BMI and embryo quality assessment, has provided further evidence to indicate the potential beneficial effects of GH supplementation in IVF treatment. Although the study has certain limitations in that it is observational and retrospective in nature, the data suggested that GH supplementation provided more live births, mainly in younger women and questions the use of adjuvant therapy in women older than 40, but particularly over 41 years. While the data does not demonstrate a significant effect on generated embryo quality, it does indicate the (+)GH may lead to more positive outcomes when embryos of lower quality are transferred. This raises the possibility the GH, whose mechanism in IVF is unknown, may influence endometrial receptivity.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

The present work was designed by JLY, KNK and SSD. Data extraction and analysis was performed by KNK, PMH and SSD. The initial manuscript draft was prepared by KNK,

and subsequently revised by JLY, SSD, AH and PMH. All the authors approved the final submitted version.

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Figure and Table Legends

Figure 1: Flow Diagram of Data Extraction

Data was extracted from the PIVET database and cases/cycles removed on the basis of, cycle outcome (e.g. cancelled/donor) and other adjuvant treatment (e.g. DHEA/Melatonin), cycle type (failed TVOA, failed fertilisation or Freeze All).

Table 1: Overview of Main Parameters that Effect Clinical Pregnancy and Live Birth Rates

Patient age was the most significant predictor of successful clinical pregnancy or live birth rates. While patients with a larger proportion of high quality embryos also had greater clinical pregnancy rates. Key parameters such as AMH level, AFC and stimulation protocol did not alter these rates.

Table 2: Overview of Main Parameters for (-)GH and (+)GH groups

From the complete data set, there was no significant difference between (+)GH cycles and (-)GH with regard to patient BMI, mean fertilisation rate, proportion of low, medium or high quality embryos generated, mean number of oocytes retrieved or mean number embryos with two pronuclei produced. However, the (+)GH group were significantly older and had a significantly lower AMH in comparision to the (-)GH group, but also had greater oocyte and embryo utilisation rates.

Table 3: Logistic Regression Analysis of Cycles

The presence of GH, patient age, transferred embryo development stage and quality were the only significant variable that affect clinical pregnancy or live birth chance. When adjusting for these variable in a multivariate logistic analysis, the effect of each parameter became stronger, as reflecte by increased odds ratios.

Table 4: Logistic Regression Analysis of Age Interaction with GH

The positive effect of GH on clinical pregnancy or live birth chance was clearly dependent on patient age. Those younger than 39 year were more likely to achieve clinical pregnancy (+)GH, than (-)GH, but (+)GH did not change the chance for those 40 and older. This was repeated for live birth chance, but those aged 40 or 41, did have a slight but significantly improved chance of live birth (+)GH.

Table 5: Logistic Regression Analysis of Transferred Embryo Quality Interaction with GH

The positive effect of GH on clinical pregnancy or live birth chance was clearly dependent on the quality of transferred embryos. (+)GH increased the clinical pregnancy when lower quality Day-3 embryos were transferred, and there was a trend towards improvements for live births with this class of embryo. However, the odds of a successful live birth were improved significantly when (+)GH was used in cycles where high quality Day-3 embryos were transferred.

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Figure 1
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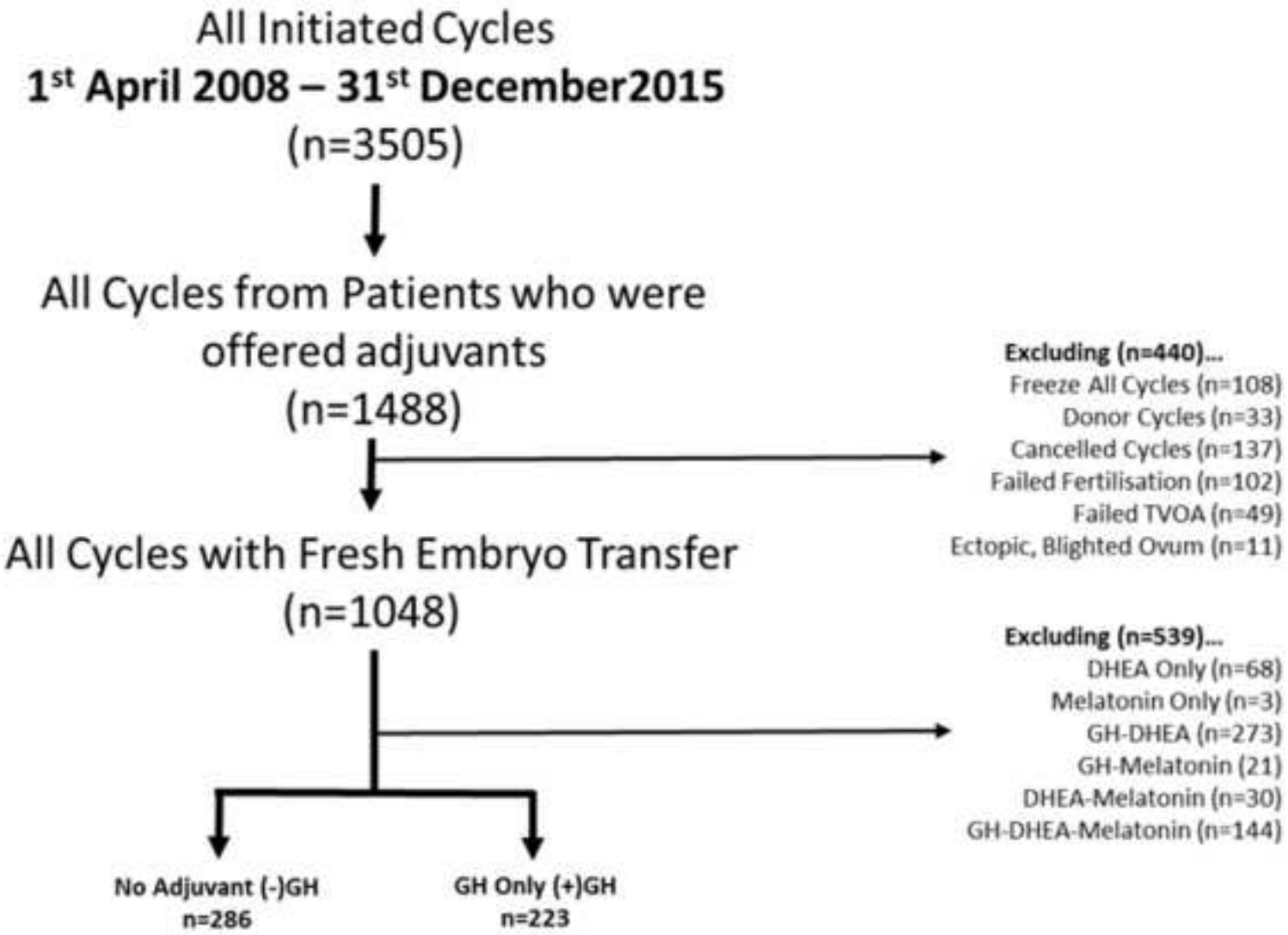

Table 1

Variable	No Clinical Pregnancy	Yes Clinical Pregnancy		Total	*p-value*
		No Live Birth	Yes LiveBirth		
Number of Cycles, N (%)	433 (85.1%)	21 (4.1%)	55 (10.8%)	509 (100%)	
Age (Years), Mean ± SD	38.9 ± 4.2	37.4 ± 3.4	36.3 ± 4.3	38.5 ± 4.2	<0.001 [a]
AMH (pmol/L), Mean ± SD	8.9 ± 11.4	12.3 ± 12.9	8.5 ± 13	9.1 ± 11.6	NS
BMI (kg/m^2), Mean ± SD	24.5 ± 4.5	26.5 ± 5.6	24.6 ± 4.3	24.6 ± 4.6	NS
Embryos Transferred (N), Mean ± SD	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	NS
Oocytes Retrieved (N), Mean ± SD	7.3 ± 4.6	8.4 ± 5	7.5 ± 4.5	7.4 ± 4.6	NS
Oocyte Utilisation Rate (%), Mean ± SD	39.0 ± 25	40.2 ± 27.2	39.3 ± 20.1	39.1 ± 24.6	NS
Two Pronuclei Generated (N), Mean ± SD	4.0 ± 3	5.2 ± 3.3	4.4 ± 2.7	4.1 ± 3	NS
Embryo Utilisation Rate (%), Mean ± SD	68.7 ± 31.7	58.3 ± 30.2	65.9 ± 27.1	68.0 ± 31.2	NS
Embryos Cryopreserved (N), Mean ± SD	0.7 ± 1.2	1.0 ± 1.3	0.9 ± 1.3	0.7 ± 1.2	NS
Fertilisation Rate (%), Mean ± SD	58.2 ± 23.8	67.6 ± 24	60.7 ± 19.1	58.8 ± 23.4	NS
High Quality Embryos Proportion (%), Mean ± SD	34.6 ± 26.7	43.0 ± 29.8	43.9 ± 21	36.0 ± 26.5	0.037 [a]
Medium Quality Embryos Proportion (%), Mean ± SD	40.2 ± 24.3	32.2 ± 23.6	38.3 ± 19.4	39.7 ± 23.8	NS
Low Quality Embryos Proportion (%), Mean ± SD	25.2 ± 24.3	24.8 ± 24.3	17.8 ± 16	24.4 ± 23.6	NS
Age Groups, N (%)					
< 35 years	70 (16.2%)	3 (14.3%)	18 (32.7%)	91 (17.9%)	
35 - 39 years	143 (33.0%)	12 (57.1%)	22 (40.0%)	177 (34.8%)	
40 - 44 years	199 (46.0%)	6 (28.6%)	15 (27.3%)	220 (43.2%)	
> 44 years	21 (4.8%)	0 (0.0%)	0 (0.0%)	21 (4.1%)	
AFC Grouping, N (%)	429	21	55		
Group A (≥ 20 follicles)	56 (12.9%)	5 (23.8%)	6 (10.9%)	67 (13.2%)	
Group B (13 - 19 follicles)	64 (14.8%)	1 (4.8%)	9 (16.4%)	74 (14.5%)	
Group C (9 - 12 follicles)	88 (20.3%)	4 (19.0%)	13 (23.6%)	105 (20.6%)	
Group D (5 - 8 follicles)	159 (36.7%)	9 (42.9%)	19 (34.5%)	187 (36.7%)	
Group E (≤ 4 follicles)	66 (15.2%)	2 (9.5%)	8 (14.5%)	76 (14.9%)	
Stimulation Protocol, N (%)					
Antagonist	196 (45.3%)	10 (47.6%)	22 (40.0%)	228 (44.8%)	
Flare Agonist	150 (34.6%)	5 (23.8%)	20 (36.4%)	175 (34.4%)	
Other (Down Regulation)	87 (20.1%)	6 (28.6%)	13 (23.6%)	106 (20.8%)	
Growth Hormone (GH) Groups, N (%)					
(-)GH	256 (59.1%)	15 (71.4%)	15 (27.3%)	286 (56.2%)	
(+)GH	177 (40.9%)	6 (28.6%)	40 (72.7%)	223 (43.8%)	

[a] *Significant difference between No Clinical Pregnancy and Yes Live Birth Groups*

[NS] No significant difference

Table 2

Variable	(-)GH	(+)GH	Total	*p-value*	
OPU Cycles, N	286	223	*509*		
Patients, N	177	194	*371*		
Age (Years), Mean ± SD	37.9 ± 4.3	39.4 ± 4.0		*< 0.001* *	A
AMH (pmol/L), Mean ± SD	10.9 ± 12.7	6.2 ± 9.0		*0.004* *	A
BMI (kg/m2), Mean ± SD	24.3 ± 4.5	24.9 ± 4.6		*0.115*	A
Oocytes Retrieved (N), Mean ± SD	7.7 ± 4.4	6.9 ± 4.8		*0.072*	A
Oocyte Utilisation Rate (%), Mean ± SD	35.0 ± 23.2	44.5 ± 25.3		*< 0.001* *	A
Two Pronuclei Generated (N), Mean ± SD	4.2 ± 3.0	3.9 ± 3.0		*0.152*	A
Fertilisation Rate (%), Mean ± SD	58.3 ± 23.7	59.5 ± 23.1		*0.559*	A
Embryo Utilisation Rate (%), Mean ± SD	62.0 ± 30.1	75.6 ± 31.0		*< 0.001* *	A
High Quality Embryos Proportion (%), Mean ± SD	35.2 ± 26.1	36.9 ± 26.9		*0.470*	A
Medium Quality Embryos Proportion (%), Mean ± SD	40.3 ± 23.6	38.9 ± 24.2		*0.533*	A
Low Quality Embryos Proportion (%), Mean ± SD	24.5 ± 23.1	24.2 ± 24.3		*0.857*	A
Fresh Embryo Transfer Cycles, N	286	223	*509*		
Fresh ET Pregnancy Rate, N (%)	30/286 (10.5%)	46/223 (20.6%)		*0.002* *	X
Fresh ET Live Birth Rate, N (%)	15/286 (5.2%)	40/223 (17.9%)		*< 0.001* *	X
Fresh ET Miscarriage Rate, N (%)	15/30 (50.0%)	6/46 (13.0%)		*< 0.001* *	X

A, T Test X, chi square Fisher's test

Table 3

Variable		Clincal Pregnancy Odds Ratio (95% CI)				Live Birth Odds Ratio (95% CI)			
		Univariate Analysis	*p-value*	Multivariate Analysis	*p-value*	Univariate Analysis	*p-value*	Multivariate Analysis	*p-value*
Growth Hormone Group	*(-)GH*	1.00	-	1.00	-	1.00	-	1.00	-
	(+)GH	2.22 (1.35 - 3.65)	0.002	2.50 (1.04 - 6.00)	0.041	3.95 (2.12 - 7.36)	0.000	5.89 (1.92 - 18.08)	0.002
Age		0.89 (0.84 - 0.94)	0.000	0.89 (0.81 - 0.98)	0.015	0.88 (0.83 - 0.94)	0.000	0.87 (0.77 - 0.98)	0.026
Serum AMH		1.00 (0.98 - 1.04)	0.715	-	-	1.00 (0.95 - 1.04)	0.823	-	-
BMI		1.03 (0.98 - 1.01)	0.239	-	-	1.00 (0.94 - 1.07)	0.922	-	-
Number of Embryos Transferred		1.17 (0.73 - 1.89)	0.509	-	-	1.24 (0.72 - 2.13)	0.444	-	-
AFC Groups	*Group A (≥ 20 follicles)*	1.00	-	-	-	1.00	-	-	-
	Group B (13 - 19 follicles)	0.80 (0.31 - 2.01)	0.629	-	-	1.41 (0.47 - 4.19)	0.539	-	-
	Group C (9 - 12 follicles)	0.98 (0.43 - 2.25)	0.969	-	-	1.44 (0.52 - 3.98)	0.486	-	-
	Group D (5 - 8 follicles)	0.90 (0.42 - 1.92)	0.778	-	-	1.15 (0.44 - 3.01)	0.776	-	-
	Group E (≤ 4 follicles)	0.77 (0.31 - 1.95)	0.583	-	-	1.20 (0.39 - 3.64)	0.753	-	-
Stimulation Protocol	*Antagonist Cycle*	1.00	-	-	-	1.00	-	-	-
	Agonist Cycle	1.02 (0.58 - 1.80)	0.943	-	-	1.21 (0.64 - 2.29)	0.563	-	-
	Other Cycle (Down Regulation)	1.34 (0.72 - 2.49)	0.359	-	-	1.31 (0.63 - 2.71)	0.469	-	-
Embryo Development Stage	*Cleavage*	1.00	-	-	-	1.00	-	-	-
	Blastocyst	2.07 (1.09 - 3.93)	0.027	-	-	1.76 (0.83 - 3.70)	0.138	-	-
Quality of Transferred Embryo	*Low Quality Day-3*	1.00	-	1.00	-	1.00	-	1.00	-
	High Quality Blastocyst	5.96 (2.49 - 14.28)	0.000	13.23 (2.89 - 60.55)	0.001	4.40 (1.62 - 12.00)	0.004	9.45 (1.64 - 54.37)	0.012
	Medium Quality Blastocyst	2.50 (0.66 - 9.56)	0.180	1.79 (0.16 - 19.46)	0.631	2.15 (0.45 - 10.42)	0.341	NC	NC
	Low Quality Blastocyst	0.83 (0.10 - 6.73)	0.865	NC	NC	1.16 (0.14 - 9.51)	0.890	NC	NC
	High Quality Day-3	2.52 (1.41 - 4.48)	0.002	3.85 (1.22 - 12.15)	0.022	2.43 (1.26 - 4.71)	0.008	3.12 (0.81 - 11.98)	0.098
Number of Infertility Factors	*None or One Factor*	1.00	-	-	-	1.00	-	-	-
	Two Factors	0.69 (0.41 - 1.17)	0.169	-	-	0.80 (0.44 - 1.46)	0.461	-	-
	Three or More Factors	0.66 (0.30 - 1.44)	0.291	-	-	0.92 (0.39 - 2.16)	0.851	-	-
Number of Previous IVF Attempts	*No Previous Attempts*	1.00	-	-	-	1.00	-	-	-
	One Previous Attempts	1.37 (0.71 - 2.63)	0.349	-	-	1.12 (0.52 - 2.42)	0.770	-	-
	Two Previous Attempts	1.08 (0.48 - 2.44)	0.856	-	-	0.84 (0.31 - 2.26)	0.732	-	-
	Three or More Previous	1.38 (0.73 - 2.62)	0.325	-	-	1.54 (0.76 - 3.13)	0.236	-	-
								-	-

NC *Not Computed due to low case number*

Table 4

Variable	No Clinical Pregnancy N (%)	Yes Clinical Pregnancy N (%)	Clinical Pregnancy Odds-Ratio (95% CI)	*p-value*	Yes Live Birth N (%)	Live Birth Odds-Ratio (95% CI)	*p-value*
	Unadjusted Analysis						
(-)GH, N (%)	256 (89.5%)	30 (10.5%)	1.00	-	15 (5.2%)	1.00	
(+)GH, N (%)	177 (79.4%)	46 (20.6%)	2.22 (1.35 – 3.65)	0.002	40 (17.9%)	3.95 (2.12 - 7.36)	< 0.000
	Analysis According to Age Group						
Age < 35 Years							
(-)GH, N (%)	53 (84.1%)	10 (15.9%)	1.00	-	7 (11.1%)	1.00	-
(+)GH, N (%)	17 (60.7%)	11 (39.3%)	3.43 (1.24 – 9.47)	0.017	11 (39.3%)	5.18 (1.74 - 15.43)	0.003
Age 35 - 39 Years							
(-)GH, N (%)	98 (89.9%)	11 (10.1%)	1.00	-	4 (3.7%)	1.00	-
(+)GH, N (%)	45 (66.2%)	23 (33.8%)	4.55 (2.05 - 10.14)	<0.000	18 (26.5%)	9.45 (3.04 - 29.39)	< 0.000
Age 40 - 44 Years							
(-)GH, N (%)	97 (91.5%)	9 (8.5%)	1.00	-	4 (3.8%)	1.00	-
(+)GH, N (%)	102 (89.5%)	12 (10.5%)	1.27 (0.51– 3.14)	0.608	11 (9.6%)	2.72 (0.84 - 8.83)	0.095
Age > 44 Years							
(-)GH, N (%)	8 (100%)	0 (0%)	NC	NC	0 (0.0%)	NC	NC
(+)GH, N (%)	13 (100%)	0 (0%)	NC	NC	0 (0.0%)	NC	NC
Age 40 or 41 Years Only							
(-)GH, N (%)	50 (89.3%)	6 (10.7%)	1.00	-	2 (3.6%)	1.00	-
(+)GH, N (%)	42 (84.0%)	8 (16.0%)	1.59 (0.51 - 4.94)	0.425	8 (16.0%)	5.14 (1.04 - 25.5)	0.045

NC *Not Computed due to low case number*

Table 5

Variable	No Clinical Pregnancy N (%)	Yes Clinical Pregnancy N (%)	Clinical Pregnancy Odds-Ratio (95% CI)	*p-value*	Yes Live Birth N (%)	Live Birth Odds-Ratio (95% CI)	*p-value*
	Unadjusted Analysis						
(-)GH, N (%)	256 (89.5%)	30 (10.5%)	1.00	-	15 (5.2%)	1.00	-
(+)GH, N (%)	177 (79.4%)	46 (20.6%)	2.22 (1.34 – 3.65)	0.002	40 (17.9%)	3.95 (2.12 - 7.36)	< 0.000
	Analysis According to Transferred Embryo Quality						
High Quality Day-3 Embryo							
(-)GH, N (%)	106 (84.8%)	19 (15.2%)	1.00	-	10 (8.0%)	1.00	-
(+)GH, N (%)	75 (76.5%)	23 (23.5%)	1.71 (0.87 - 3.36)	0.119	21 (21.4%)	3.14 (1.40 - 7.02)	0.005
Low Quality Day-3 Embryo							
(-)GH, N (%)	116 (95.9%)	5 (4.1%)	1.00	-	4 (3.3%)	1.00	-
(+)GH, N (%)	90 (86.5%)	14 (13.5%)	3.61 (1.25 - 10.39)	0.017	10 (9.6%)	3.11 (0.95 - 10.24)	0.062

*Completed STROBE checklist

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study Design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	
		Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	4 & 5
		Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	
		Case-control study—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6 & 7

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6&7
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	4&5
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4/5/6
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6&7
		(*b*) Describe any methods used to examine subgroups and interactions	6&7
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	~~6&7 4&5~~ 4&5
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4&5
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	Figure 1
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1+2
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	4&5
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-10
		(*b*) Report category boundaries when continuous variables were categorized	Table 1-5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9+10
Discussion			
Key Results	18	Summarise key results with reference to study objectives	10-13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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