A direct action for GH in improvement of oocyte quality in poor-responder patients

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Abstract

Declining female fecundity at later age and the increasing tendency for women to delay childbirth have lead to a drastic rise in the number of women seeking assisted reproductive technology. Many women fail to respond adequately to standard ovarian stimulation regimens, raising a significant therapeutic challenge. Recently, we have demonstrated that the administration of GH, as an adjunct to ovarian stimulation, has improved the clinical outcomes by enhancing the oocyte quality. However, the mechanism(s) by which GH facilitated this improvement is yet to be understood. This study aimed to determine these potential mechanism(s) through the use of immunofluorescent localisation of GH receptors (GHRs) on the human oocyte and unbiased computer-based quantification to assess and compare oocyte quality between women of varying ages, with or without GH treatment. This study demonstrates for the first time, the presence of GHRs on the human oocyte. The oocytes retrieved from older women showed significant decrease in the expression of GHRs and amount of functional mitochondria when compared with those from younger patients. More interestingly, when older patients were treated with GH, a significant increase in functional mitochondria was observed in their oocytes. We conclude that GH exerts a direct mode of action, enabling the improvement of oocyte quality observed in our previous study, via the upregulation of its own receptors and enhancement of mitochondrial activity. This result, together with recent observations, provides scientific evidence in support of the use of GH supplementation for the clinical management of poor ovarian response.

Introduction

Since the development of modern effective forms of contraception, an ever increasing number of women are choosing to postpone pregnancy until later life, for various reasons including, but not limited to, a dedication to their chosen profession, level of education, and/or given lifestyle (te Velde & Pearson 2002, Broekmans *et al*. 2007). As such, the modern tendency for women to defer pregnancy has lead to a sharp rise in the mean female age at first childbirth (Perheentupa & Huhtaniemi 2009). Unfortunately, by delaying childbirth beyond the second decade of reproductive life (late 30s), the probability of successful conception is drastically reduced, hence increasing both the incidence of age-related subfertility and the number of advanced reproductive age women (≥35 years) seeking fertility assistance (Broekmans *et al*. 2007).

Current prevailing concepts of female reproductive ageing attribute declining female fecundity to the parallel diminishment in both the quantity and quality of the primordial follicle reserve and oocytes respectively (te Velde & Pearson 2002, Perheentupa &

Huhtaniemi 2009). Whilst the complete exhaustion of the ovarian reserve ultimately results in menopause, diminishing reproductive potential during the pre-menopausal period is attributed primarily to the compromised quality of oocytes (Madankumar *et al*. 2003). However, little is understood regarding the exact cellular mechanism(s) and cause(s), which facilitate the decline in oocyte quality.

The successful outcome of IVF is largely dependent upon adequate patient response to ovarian stimulation, as it determines both the number and quality of oocytes available for clinical treatment. Unfortunately, ∼9–24% of all IVF cycles undertaken fail to respond to standard ovarian stimulation regimens, due to poor ovarian response (POR) to hormonal stimulation (Abir *et al*. 2008). Currently, the management of POR patients represents a significant therapeutic challenge (Hazout *et al*. 2009, Yovich & Stanger 2010), such that various alternative forms of clinical intervention have either been ineffective or lack sufficient scientific evidence necessary to support clinical application (Ubaldi *et al*. 2005). Recently, we have demonstrated that the administration of exogenous growth hormone (GH)

supplementation, in conjunction with standard ovarian stimulation regimens, resulted in significantly higher embryo implantation and clinical pregnancy rates compared with those not treated with GH (Yovich & Stanger 2010). These findings are supported by previous studies demonstrating the beneficial effect of GH in increasing the response of human ovary to gonadotrophins in poor-responder patients (Homburg *et al.* 1988, Volpe *et al.* 1989, Hazout *et al.* 2009). The improvement in clinical outcomes was likely achieved via an improvement in oocyte developmental competence, rather than the result of an increased yield of oocytes or effects on the endometrium as the benefits continued to express in subsequent frozen embryo transfer cycles.

At present, very little is known regarding the potential mechanisms(s) by which GH improves oocyte developmental potential. However, a recent study conducted in our laboratory has demonstrated that GH may indirectly achieve this improvement by promoting granulosa cell responsiveness to gonadotrophin stimulation, reflected by an increase in the expression of receptors to follicle-stimulating hormone (FSH) and luteinising hormone (LH) (Regan *et al.* 2012). Although it is plausible that GH may also elicit a direct effect upon the oocyte itself, the existence of GH receptors (GHRs) upon the human oocyte retrieved from IVF patients has yet to be confirmed, and the potential direct mechanism by which GH improves oocyte quality remains to be shown.

Non-invasive selection of developmentally competent human oocytes is of particular importance within modern fertility treatments (Rienzi *et al.* 2011). Typically, gross morphological assessment of denuded oocytes or oocyte–cumulus complex is evaluated using simple, non-invasive light microscopy in order to ascertain oocyte quality (Rienzi *et al.* 2011) and its developmental potential (Balaban & Urman 2006). However, the integrity of important intra-cellular organelles and ultra-structures such as mitochondria and microtubules cannot be accurately determined through such gross morphological assessment, hence concerns have been raised regarding the predictive value of these parameters (Rienzi *et al.* 2011). Instead, we have demonstrated that more detailed, non-invasive biochemical and/or ultra-structural evaluation is necessary to adequately assess true oocyte developmental competence (Seet *et al.* 2013).

Mitochondria are the most abundant organelles within oocytes, and although oocytes are relatively less metabolically active, their function is essential for oocyte/embryo development (Cummins 2004). Sufficient levels of mitochondrial activity are necessary to facilitate the high energy-demanding processes of the oocyte, including spindle formation, chromosomal segregation and meiotic division, fertilisation and embryonic division (Cummins 2004, Eichenlaub-Ritter *et al.* 2011). In fact, the age-dependent decline in oocyte quality is predominantly due to meiotic non-disjunction and consequently, potentially fatal aneuploidies (Broekmans *et al.* 2007).

The number of mitochondria present in an oocyte may indicate the energetic status of the oocyte, which has been the aim for several investigations using different approaches and markers for estimating their numbers and/or mass quantity such as cytochrome *c* oxidase (Duran *et al.* 2011, Eichenlaub-Ritter *et al.* 2011). However, mitochondria are in a consistent dynamic turnover because of their short functional life span (Cummins 2004). At any given time, several mitochondria could be visibly counted in an oocyte but not necessarily are all functional, they remain visible for a while before they degenerate and disappear. As such, it is possible that the number of mitochondria in older women may show no difference when compared with those found in the oocyte of younger women, but they may not be fully functional. In order to properly estimate the developmental potential of the oocyte, both the functional capacity and structural integrity of mitochondria should be investigated.

This study aimed to investigate and determine the presence of the potential mechanism(s) by which GH supplementation, in conjunction with standard ovarian stimulation, improves oocyte developmental competence. Specifically, this was achieved through the application of an unbiased immunofluorescent labelling and computer-based quantification technique to localise GHRs on the human oocyte and to assess and compare oocyte quality between women of varying ages, with and without GH treatment. To ascertain true oocyte developmental potential, fluorescence and immunofluorescence labelling were used to quantify the mitochondrial structural integrity and functional viability, in a manner previously described by Seet *et al.* (2013). The findings were then compared against the findings of a traditional, non-invasive form of oocyte quality assessment for evaluating the validity of these prognostic tests.

Materials and methods

Patient recruitment and data collection

Cumulus–oocyte complexes (COCs) were collected from women aged between 26 and 46 years (mean 30 years), undergoing fertility treatments at PIVET Medical Centre. The cause(s) of infertility were various, being female or male derived, a combination of both or unexplained. All patients underwent routine cycle stimulation using a gonadotrophin-releasing hormone (GNRH) antagonist (Orgalutran, MSD, North Ryde, NSW, Australia) plus recombinant FSH (Puregon, MSD) regimen, at customised dosages, as described by the PIVET Clinical Algorithm (Yovich *et al.* 2012), which effectively avoids ovarian hyperstimulation syndrome. Women deemed susceptible to POR were given the option of receiving exogenous GH supplementation in conjunction with their prescribed stimulation regimen. Identification of

'poor-responder' cases was based on the patient's fulfilment of one or more of the following 'Bologna' criteria (Ferraretti *et al.* 2011): i) an antral follicle count of ≤5; ii) a circulating anti-Mullerian hormone level of ≤5 pmol/l; iii) the generation of ≤3 mature oocytes at the time of collection, despite receiving the maximal allowed gonadotrophin dosage (i.e. ≥450 IU/day); and iv) repeated failure to achieve clinical pregnancy following the transfer of fresh and/or frozen embryos, where poor oocyte and/or embryo quality has been noted.

GH supplementation was performed using either Saizen injection, an average of 2.5 IU/day for 24 days (Merck Serono) or SciTropin A pen at 1 IU/day for 4–6 weeks (SciGen, Belrose, NSW, Australia). To avoid any possibility of bias, the selection of patients who did or did not choose to undertake GH administration, the study was designed as a sequential crossover (Yovich & Stanger 2010), where patients identified as poor responders were given the option of taking GH or await the outcome of the treatment cycle then using GH on the next cycle if the first failed.

Ovulation was triggered by the timed administration of two injections of Ovidrel (each 250 mg/0.5 ml equating to ∼6500 IU dosages of recombinant human CG; Ovidrel, Merck Serono). Trans-vaginal oocyte recovery was undertaken 36 h thereafter.

Human oocytes

All retrieved COCs were graded by an embryologist, as initially described (Marrs *et al.* 1984) with modifications (Yovich & Grudzinskas 1990) for the initial selection process of oocytes for ICSI. In brief, grading was based upon the extent of cumulus expansion within the COC. The COCs of the highest grade exhibited a high level of cumulus expansion denoted by widely dispersed luteinised cumulus cells in a large loose mass. The coronal cells are also somewhat dispersed in a radiant 'sunburst' pattern, enabling reasonable visualisation of the oocyte. Lower grade COCs display tighter, smaller and undispersed cumulus masses with a dense circular coronal coat containing a dark, essentially non-visible oocyte. All COC grades were included into this study.

The oocytes were denuded using 1500 IU of hyaluronidase (Hyalase, Sanofi Aventis, Macquarie Park, NSW, Australia), ∼1–2 h after collection, and stored in a fertilisation medium at 37 °C with 5% CO_2, 5% O_2 and 90% N_2 until ICSI. All MII oocytes were selected for ICSI, while those identified as immature (MI or germinal vesicle (GV) oocytes) were kept in incubator for 4–6 h. Some MI oocytes matured *in vitro* to MII oocytes and could be used as a back-up reserve for clinical purpose. Once fertilisation and formation of good embryos were achieved, the remaining MI and/or MII oocytes superfluous to clinical need were donated to the study. In total, 149 women aged between 27 and 46 years gave written informed consent to donate their unused oocytes, based on ethical approval from Curtin University Human Research Ethics Committee. The oocytes were allocated into groups according to patients' age and classified according to whether GH supplementation had been given or not. They were randomly selected for either GHR immunofluorescence localisation or the assessment of mitochondrial integrity and function.

Immunofluorescent labelling

The following procedures were performed on fresh oocytes in a progressive way, while the oocytes were collected. At the end of the study period, all collected data were subjected to statistical analysis. An indirect immunofluorescence staining was used, as described previously (Almahbobi & Hall 1993, Seet *et al.* 2013), to localise GHR. In brief, oocytes were fixed in 4% paraformaldehyde (PFA) and incubated overnight in 4 μg/ml goat anti-human GHR antibody (R&D Systems, Minneapolis, MN, USA) at 4 °C. After washing in PBS, the oocytes were incubated in 4 μg/ml donkey anti-goat IgG antibody conjugated to Alexa 488 (Sigma–Aldrich) for 45 min at room temperature.

For mitochondrial assessment, oocytes were pre-labelled using 100 nM MitoTracker Red CMX (Molecular Probes, Eugene, OR, USA) in PBS for 30 min at 37 °C with 5% CO_2 (Stojkovic *et al.* 2001). This fluorescent marker with an excitation wavelength of 594 nm labels functional mitochondria in live cells, dependent upon membrane potential for the evaluation of mitochondrial viability and distribution. After washing in PBS, oocytes were fixed in 4% PFA before permeabilisation, using 0.02% Triton X-100. Subsequently, the samples were incubated overnight at 4 °C in 2 μg/ml mouse anti-cytochrome *c* oxidase MAB (Santa Cruz Biotechnology) followed by incubation in 4 μg/ml goat anti-mouse IgG secondary antibody (Molecular Probes) conjugated to Alexa 488, for 45 min at room temperature. The samples were mounted using an anti-fade mounting medium, containing 4′,6′-diamidino-2-phylindole (Molecular Probes). For the negative controls of immunofluorescent labelling, oocytes were incubated in pre-immune goat or mouse serum diluted at 1:10 (v:v) in PBS instead of the corresponding primary antibodies.

Confocal microscopy and quantification

The oocytes were examined using an inverted confocal microscope (AR1+/A1+; Nikon Corporation, Tokyo, Japan), equipped with a 40× objective and 488 and 594 nm filters. Methods for image capture and 3D signal quantification were employed as described previously (Seet *et al.* 2012) with slight modifications. Briefly, equatorial serial sections were captured throughout the entire length of the oocyte, at 2 μm intervals. The fluorescent emission intensity of the reconstructed 3D image, resulting from a stacking of these serial sections, was quantified using Volocity 3D image analysis software, version 6.2 (Perkin Elmer, Waltham, MA, USA). In GHR-labelled oocytes, signal emission was measured in four 5 μm^3 regions around the cell membrane in three equatorial serial sections located at the centre of the oocyte, where the nucleus was visible. This method of measurement was applied consistently to all GHR-labelled oocytes. The mean value of the emitted signals from these four points was used to reflect the level of GHR expression for a given oocyte. In oocytes labelled for MitoTracker and cytochrome *c* oxidase, total fluorescent emission was measured in all 2 μm equatorial serial sections throughout the entire oocyte. All above procedures were

performed blindly without reference to the patient group from which the oocytes were derived.

Statistical analyses

Oocyte and COC morphological grade distribution was analysed with JMP version 10 (SAS Institute, Cary, NC, USA) statistical analysis software, using one-way ANOVA non-parametric Wilcoxin t-tests, with statistical significances set at $P < 0.05$. Immunofluorescence results were analysed using Prism version 5 (GraphPad Software, La Jolla, CA, USA) statistical analysis software, one-way ANOVA and Student's t-tests with statistical significances set at $P < 0.05$. One-way ANOVA tests were used to compare all groups, whilst Student's t-tests were used to analyse differences between individual groups.

Results

Assessment of COC

The distribution of COC morphological grades, according to the different patient groups, can be seen in Fig. 1a and b. In both age groups, normo-responder patients produced a significantly greater number of good quality oocytes (grade 2.5; $P < 0.05$) compared with the age-matched poor-responder counterparts. Interestingly, poor-responder women treated with GH produced more grade 2.5 good quality oocytes compared with those not treated with GH. However, the number of good quality oocytes recovered from poor-responder GH-treated women was still considerably lower than those in normo-responder patients and consequently the improvement trend was not statistically significant in either age group (Fig. 1a and b).

In young patients only, oocytes of other lesser quality grades showed a trend of difference in their distribution according to patient response and treatment, particularly observed in grades 1.5 and 2. The number of grade 1.5 oocytes significantly increased in untreated poor-responder patients compared with age-matched normo-responders, but then reduced after GH treatment (Fig. 1a). Grade 2 good quality oocytes showed significant increase in number in GH-treated poor-responder patients when compared with their age-matched untreated counterparts (Fig. 1a). No such correlation has ever been observed in older patients (Fig. 1b).

Immunofluorescence localisation and quantification of GHR

Positive immunofluorescent labelling (Fig. 2a) of GHR was detected on the surface of human oocytes (Fig. 2c). In negative controls, no fluorescent signal was detected (Fig. 2b). 3D quantitative analysis of the level of GHR expression, as reflected by fluorescent signal intensity, demonstrated a considerable disparity in receptor

(a)

(b)
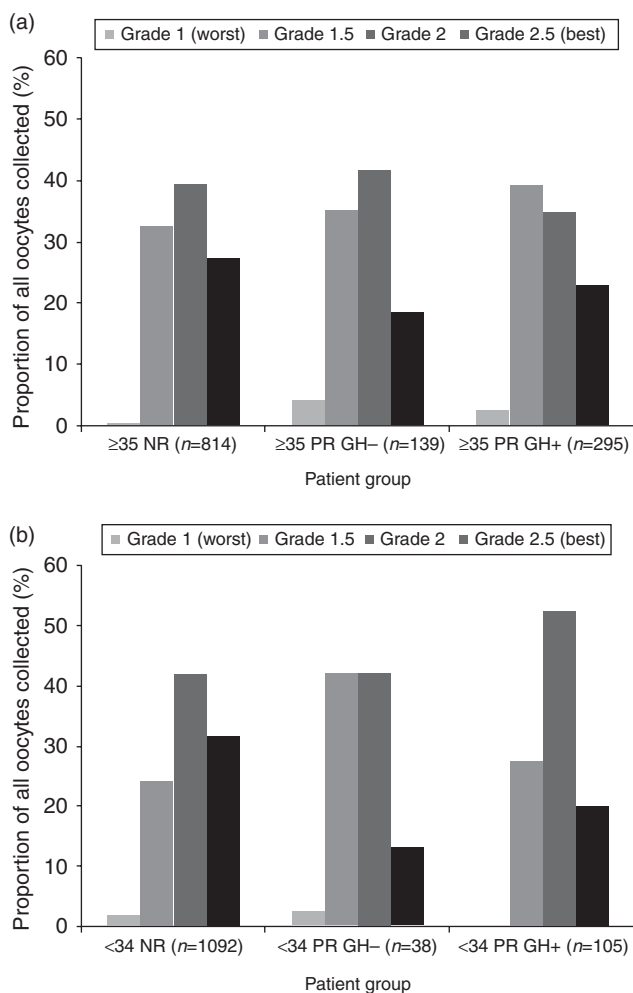
Figure 1 Distribution of COC morphological grade, according to patient age and treatment. (a) COC grade distribution in older patients ≥35 years. (b) COC grade distribution in younger patients <35 years. Grading was based upon extent of cumulus expansion within the COC. NR, normo-responder; PR, poor-responder; GH−, untreated; GH+, treated; n, number of oocytes.

expression in oocyte recovered from women of varying ages (Fig. 2d). The oocytes recovered from younger normo-responder women (<35 years of age) exhibited a significantly higher level of GHR expression ($P < 0.04$) compared with those recovered from older normo-responder women of ≥35 years (Fig. 2d).

Assessment of mitochondrial integrity and viability using fluorescent microscopy

Confocal microscopic observation revealed a homogenous similar distribution of MitoTracker Red (Fig. 3b) and cytochrome c oxidase (Fig. 3c) fluorescent labelling throughout the cytoplasm. Dual visualisation of both fluorescent labels, using image super-imposition, demonstrated perfect co-localisation of the two labelling (Fig. 3d). In negative controls, no fluorescent signal could be detected (data not shown), as in Fig. 1b.
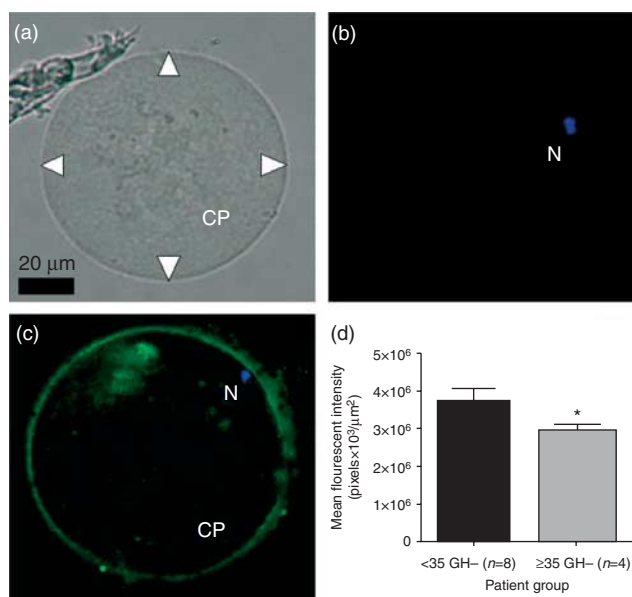
Figure 2 Localisation and quantification of GHR on human oocytes. (a) Light microscopic image of an immature oocyte showing the four regions, denoted by the arrowheads, where quantification of GHR was performed. (b) Negative control exhibiting no detectable immuno-labelling. (c) Immunofluorescent labelling of an oocyte demonstrating cell membrane-bound GHR expression and distribution. (d) Quantification of GHR expression in fresh oocytes taken from women younger and older than 35 years. Data shown is the mean value±s.e.m. A significant difference was found, as indicated by *($P<0.05$). CP, cytoplasm; GH−, GH untreated patients; GH+, GH treated patients; N, nuclear material; n, number of oocytes; *$P<0.05$.

3D quantification of mitochondria integrity and viability in different patient groups

3D quantification of MitoTracker Red labelling demonstrated a significant difference in the amount of viable mitochondria between each of the patient groups (Fig. 4a). The oocytes recovered from younger normo-responder women had a significantly ($P<0.001$) larger amount of functional mitochondria, than those taken from both older normo- and poor-responder women. More interestingly, oocytes recovered from the older poor-responder GH-treated women had a significantly ($P<0.005$) higher level of viable mitochondria, compared with their age-matched counterparts, untreated with GH. Although the level of functional

mitochondria in poor-responder patients treated with GH was considerably lower than that observed in younger normo-responder patients, it was not statistically significant probably due to small number of patient ($n=3$; one treated with Saizen, two with SciTropin A). The total number of mitochondria, as reflected by the extent of cytochrome *c* oxidase labelling, was comparable between each of the patient groups and was not statistically significant (Fig. 4b).
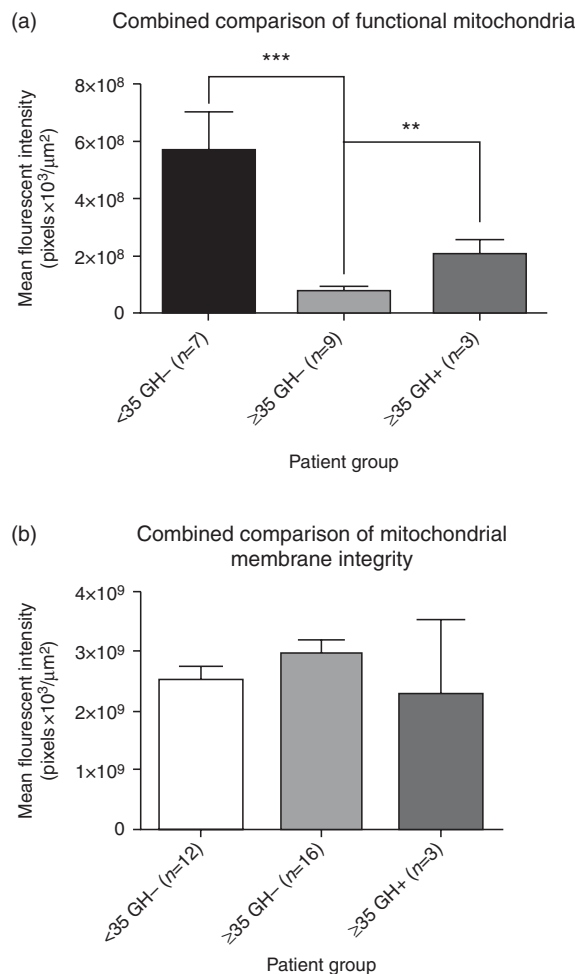
Discussion

This study provides further understanding of the role of GH in female reproduction, supporting the clinical administration of exogenous GH for the management of POR. This was achieved through the application of fluorescence and immunofluorescence labelling and computer-based unbiased 3D quantification, as we have previously reported (Seet *et al.* 2013, Al-Samerria & Almahbobi 2014).

A growing body of evidence, in both human and animal models, suggests that GH is an important regulator of ovarian steroidogenesis (Nakamura *et al.* 2012), follicular development (Bachelot *et al.* 2002) and oocyte maturation (Bevers & Izadyar 2002). In clinical application, we have recently demonstrated that GH supplementation together with gonadotropin-induced ovarian stimulation has lead to significant improvement in oocyte quality (Yovich & Stanger 2010). However, it is not yet clear how GH exerts its action on human oocytes. In another study conducted in our laboratory, we have demonstrated that GH administration to IVF patients significantly increases the expression of both FSH and LH receptors on granulosa cells, suggesting an indirect mode of action on human oocytes via granulosa cells (Regan *et al.* 2012).

In this study, we report for the first time the presence of immunoreactive cell membrane-bound GHR on human oocytes retrieved from IVF patients, suggesting that GH may elicit a direct effect upon the oocyte itself. This may appear in contrast with previous reports showing the absence of *GHR* mRNA in human (Sharara & Nieman 1994) and mouse (Terada *et al.* 1996) oocytes. However, GHR protein was expressed in bovine (Bevers & Izadyar 2002) and rat (Zhao *et al.* 2002) oocytes, even when the
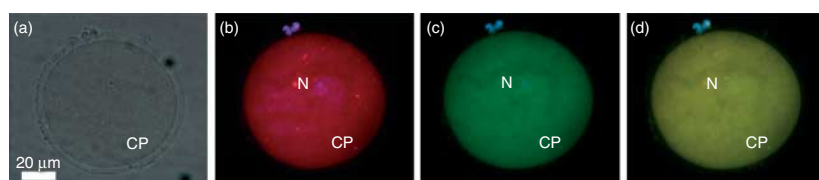
Figure 3 Fluorescent and immunofluorescent labelling of mitochondria and their distribution. (a, b, c and d) Microscopic images of the same oocyte. (a) Light microscopy. (b) Viable mitochondria labelled by MitoTracker Red. (c) Total amount of mitochondria immunolabelled by cytochrome c oxidase. (d) Dual-labelled image of MitoTracker Red and cytochrome *c* oxidase demonstrating perfect co-localisation. CP, cytoplasm; N, nuclear material; PB, polar body.

(a)

Combined comparison of functional mitochondria

(b)

Combined comparison of mitochondrial membrane integrity

Figure 4 Quantification of immunofluorescent labelling of mitochondria in oocytes taken from women younger and older than 35 years, with and without GH. (a) Assessment of mitochondrial viability based on MitoTracker Red fluorescent labelling. (b) Assessment of mitochondrial membrane integrity, based on cytochrome *c* oxidase immunolabelling. Data shown is mean value ± s.e.m. Significant differences were found, as indicated by asterisks, using *t*-tests. *n*, number of oocytes; GH−, GH untreated patients; GH+, GH treated patients. **$P < 0.005$ and ***$P < 0.001$.

GHR mRNA was reported to be absent in rat oocytes (Zhao *et al*. 2002). Nonetheless, *in situ* expression of GHR protein and mRNA was detected in human oocytes, although the signals were found in the cytoplasm (Abir *et al*. 2008). Taken into consideration such a confusing discrepancy in the literature, it appears that GH action on oocyte may be mediated by species-specific pathways. The presence of GHR protein in rat (Zhao *et al*. 2002) and human (Abir *et al*. 2008, present study), while the GHR mRNA was not detected in these oocytes (Sharara & Nieman 1994, Zhao *et al*. 2002, respectively), requires further investigation.

Quantitative analysis revealed an age-dependent decline in GHRs expressed in the oocyte, which to the best of our knowledge has not been reported previously. These results support our previous report,

confirming an age-dependent decline in GHR expression within the granulosa cells of human Graafian follicles (Regan *et al*. 2012). In addition, granulosa cells retrieved from women who received GH supplementation expressed a significantly higher level of GHR protein than those from age-matched counterparts (Regan *et al*. 2012) and *GH* mRNA expression in the ovaries of hypophysectomised rat was significantly increased after treatment with GH (Carlsson *et al*. 1993), suggesting that GH upregulates its own receptor expression. We believe that this auto upregulatory action of GH may also be present in the oocyte, which requires further investigation.

This study also reports that reproductive ageing leads to a decrease in the amount of functionally viable mitochondria in human oocytes, thereby contributing to the age-related decay in oocyte developmental competence. As mitochondrial function is essential for both oocyte and embryonic development (Cummins 2004, Eichenlaub-Ritter *et al*. 2011), it appears that the significantly reduced functional mitochondria in the oocytes recovered from women of advanced reproductive age underlines the impairment of oocyte developmental competence (Madankumar *et al*. 2003) by bioenergetic deficiencies, resulting in chromosomal segregation disorders (Schon *et al*. 2000), failed maturation and fertilisation (Reynier *et al*. 2001), and arrested cellular development (Van Blerkom 2011). However, our observation contrasts with a previous study (Duran *et al*. 2011), which may be due to a difference in the mitochondrial assessment technique used.

Most interestingly, the current study demonstrated that the administration of GH, as an adjunct to standard ovarian stimulation, significantly increased oocyte mitochondrial function, based on MitoTracker Red labelling. This is similar to a recent report that acute GH action promotes mitochondrial oxidative capacity within skeletal muscle cells (Short *et al*. 2008). We also found that GH did not promote the formation and/or growth of mitochondria contained within the oocyte, as evidenced by the level of cytochrome *c* oxidase labelling. Instead, it has been suggested that GH action may activate various proteins in the β-oxidation or tricarboxylic acid cycles, or other components of the mitochondrial fuel delivery and oxidative machinery, thereby enhancing the capacity of oxidative ATP generation (Short *et al*. 2008). However, the exact mechanism through which GH action regulates oocyte mitochondrial function remains to be elucidated, along with considerations related with the GH dosage; in this study being either 1 IU or 2.5 IU daily. Nevertheless, we can thus confirm that the administration of exogenous GH supplements, in conjunction with standard ovarian stimulation regimens, does indeed improve the quality of human oocytes, as demonstrated previously (Yovich & Stanger 2010), via a potential enhancement in mitochondrial functionality. With respect to older women, improvements in pregnancy rates have been clearly

demonstrated using 8 IU daily for a short interval; from day 7 for 6–8 days to the day after Trigger (Tesarik *et al.* 2005) along with our studies where the Saizen regimen produced comparable improvements (Yovich & Stanger 2010). The SciTropin A regimen has also shown equivalent clinical benefits in other study (JL Yovich and J Stanger, unpublished observations).

Our results demonstrate that reduced numbers of good quality oocytes, reflected by high-grade COCs, can be used as a marker for reproductive ageing. One of the essential precursory steps before ovulation is the dissociation of the cumulus cells surrounding the oocyte, indicated by the COC grades 2/2.5. More interestingly, COC grading did appear to show a general improvement in the quality of oocytes retrieved from poor-responder women treated with GH, compared with their age-matched poor-responder counterparts. This outcome can be explained by the presence of GHR on human granulosa cells (Sharara & Nieman 1994) and the significant increase in the levels of FSH and LH receptors observed in granulosa cells of human preovulatory follicles after GH treatment (Regan *et al.* 2012). Nonetheless, we acknowledge that multiple studies have demonstrated that there is no correlation between COC morphology and fertilisation, embryo cleavage and clinical pregnancy rates (Rattana-chaiyanont *et al.* 1999, Rienzi *et al.* 2011).

In conclusion, this study demonstrates for the first time the presence of cell membrane-bound GHR on the human oocyte collected from IVF patients, thus enabling a potential direct mode of action by which GH may improve oocyte developmental competence in patients with POR. The action of GH is mediated via the promotion of GHR expression and functional viability rather than the numbers of mitochondria. However, the exact mechanism(s) by which GH action improves mitochondrial viability in the oocytes remains to be investigated. Treatment with GH also resulted in a significant shift in favour of good quality grades of the overall COC. We conclude that diminished GHR expression and mitochondrial activity may be the significant contributing factors to the decline in oocyte developmental potential associated with reproductive ageing. These results, in conjunction with recent observations (Regan *et al.* 2012), provide further scientific evidence in support of the use of exogenous GH supplementation for the clinical management of POR.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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