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Evidence Based Women’s Health Journal

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References: References should be numbered consecutively in the order in which they first appear in the text. They should be assigned Arabic numerals, which should be given in brackets, e.g. [17]. References should include the names of all authors when six or fewer; when seven or more, list only the first six names and add et al. References should also include full title and source information. Journal names should be abbreviated as in MEDLINE (NLM Catalog, http://www.ncbi.nlm.nih.gov/nlmcatalog).
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Mobile: + 0111 222 0298
E-mail: kaainih@yahoo.com
Evidence Based Women’s Health Journal

Focus and Scope

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Does the addition of LH activity to FSH make gonadotrophins more superior? A systematic review and meta-analysis
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Introduction
Gonadotrophins differ in terms of the source from which they are extracted (either from urine or from recombinant DNA technology) and in their formulation [either containing both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) or either FSH or LH only]. The composition as well as the source of gonadotrophins could have an impact on the outcome of IVF. Human menopausal gonadotropins (hMG) have long been the classic and principal urinary gonadotrophins used for ovarian stimulation since the development of ART technology in the 1970s. It is composed of equal amounts of FSH and LH (75 U for each per ampoule). However, since the 1980s, a variety of generations of urinary hMG have been developed with the intention of eliminating most or all of the LH content [1]. During the mid-1990s, recombinant FSH (recFSH) was produced from hamster ovarian cell cultures and this step was considered a landmark in the production of gonadotrophins [2].

With the development of such gonadotrophins devoid of LH activity (namely recFSH and HP-FSH), questions about the role of adding LH activity to FSH in controlled ovarian hyperstimulation (COH) in IVF and/or intracytoplasmic sperm injection (ICSI) on treatment outcome have been raised and have been a topic for intense research.

Previous systematic reviews have primarily focused on comparing the effectiveness of different sources of gonadotrophins. Urinary hMG was compared with recFSH in two recent meta-analyses, both of which showed...
a better outcome in terms of live birth rate when hMG was used for ovarian stimulation compared with recFSH in the GnRH agonist (GnRHa) long protocol [3,4]. Two other meta-analyses compared urinary HP-FSH versus (recFSH) and showed similar pregnancy and live birth rates, with no difference in the ovarian hyperstimulation (OHSS) rate [5,6].

However, there is still a need for a better understanding of the differential effects of different gonadotropin preparations and of the impact of the administration of LH activity during COH.

To extract the evidence on the impact of COH with LH-containing gonadotrophins on live birth rate and OHSS rate, the current meta-analysis and systematic review was carried out to compare FSH-only protocols (recFSH) with LH-containing protocols irrespective of the source of gonadotrophins (either hMG or recFSH + recLH) and irrespective of the protocol of desensitization (either the GnRHa long protocol or the GnRH antagonist protocol).

Materials and methods
Criteria for considering studies for this review
All published manuscripts and abstracts describing randomized trials and reporting data that compared outcomes for women undergoing IVF/ICSI and randomized to undergo ovarian stimulation with FSH-only protocols (recFSH) or LH-containing protocols (either hMG or recFSH + recLH) were sought in all languages.

Search strategy for the identification of studies
Meticulous computerized searches (last performed...) were carried out using MEDLINE (1978 to present), EMBASE (1980 to present), the Cochrane Central Register of Controlled Trials (CENTRAL), and the Trial Register of Controlled Trials (e.g. http://www.controlled-trials.com). Furthermore, the reference lists of all known primary studies, review articles, citation lists of relevant publications, abstracts of major scientific meetings (e.g. ESHRE and ASRM), and included studies were examined to identify additional relevant citations. Finally, ongoing and unpublished trials were sought by contacting experts in the field and commercial entities.

Methods of the review
A standardized data extraction form was developed and piloted for consistency and completeness. Trials were considered for inclusion and trial data were extracted. Data management and statistical analyses were carried out using the 'Review Manager 4.3' (The Cochrane Collaboration). Individual outcome data were included in the analysis if they fulfilled the prestated criteria. Where possible, data were extracted to allow for an intention-to-treat analysis, defined as including all randomized cycles in the denominator. If data from the trial reports were insufficient or missing, the investigators of individual trials were contacted by e-mail for additional information to carry out analysis on an intention-to-treat basis.

For the meta-analysis, the number of participants experiencing the event in each group of the trial was determined by visual inspection of the outcome tables and using the $\chi^2$-test for heterogeneity. In addition, the $F^2$-test was used to attempt quantification of any apparent inconsistency. The $F^2$-test is a statistical measure used to quantify heterogeneity. It describes the percentage of the variability in effect estimates that is because of heterogeneity rather than sampling error (chance). An $F^2$-value greater than 50% may be considered to represent substantial heterogeneity.

Types of outcomes measures
The primary outcomes for this systematic review were live birth rate and OHSS rate. The secondary outcomes were clinical and ongoing pregnancy rates. In addition, cycle characteristics (number of oocytes retrieved, treatment duration, amount of FSH, estradiol on day of hCG, progesterone on day of hCG, rate of poor responders) were also evaluated. The trials included were analyzed for the following characteristics: method of randomization, presence or absence of blinding to treatment allocation, quality of allocation concealment, number of patients randomized, whether an intention-to-treat analysis was carried out, and the duration, timing, and location of the study.

Comparison of results
For the meta-analysis, the number of participants experiencing the event was recorded. Data were extracted to allow for an intention-to-treat analysis, defined as including in the denominator all randomized cycles. Meta-analysis of dichotomous data was carried out using the Mantel–Haenszel method using a random-effects model, and the odds ratio (OR) and 95% confidence interval (CI) were evaluated. Meta-analysis of continuous data was carried out using the inverse variance method utilizing a random-effects model. The mean difference (MD) and 95% CI were evaluated. $P$ values were presented for further confirmation of the results. The standardized mean difference was used when multiple scales were provided (E2). A $P$ value of less than 0.05 was considered to be significant. The results were pooled using a fixed-effects model only after confirming that marked statistical heterogeneity was not present (i.e. the observed treatment effects in individual trials were not statistically significantly different from the overall pooled estimate of the treatment effect).

Participants
Randomized women undergoing IVF/ICSI and COH with either FSH-only protocols (recFSH) or LH-containing protocols (either hMG or recFSH + recLH). We excluded all nonrandomized trials, trials using LH priming (LH only then FSH only), and trials using HP-FSH instead of recFSH (as the HP-FSH contains some LH activity).
Results
Thirty-one randomized-controlled trials (RCTs) were identified. Twenty-one trials compared the use of recFSH vs. hMG/H-P-hMG whereas 10 RCTs compared recFSH vs. recFSH + recLH. The method of fertilization used was either IVF or ICSI. These included trials enrolled 4571 participants, which yielded an adequate power for meta-analysis.

Nine trials only reported on live birth rate and OHSS rate. Data were extracted to allow for an intention-to-treat analysis. Pooling of the included trials showed that live birth rate was not statistically different between both groups, although it showed trends toward improvement with LH-containing protocols [recFSH (304/1120; 27.14%) vs. FSH/LH (324/1110; 29.19%) (P = 0.29; OR = 0.90, 95% CI = 0.75–1.09)] (Fig. 1).

The rate of OHSS was also not statistically different between FSH-only protocols and LH-containing protocols [recFSH (34/1888; 1.80%) vs. FSH/LH (29/1843; 1.57%) (P = 0.79; OR = 1.08, 95% CI = 0.63–1.83)] (Fig. 2).

Eighteen trials assessed the ongoing pregnancy rate, which showed no significant difference between both groups [recFSH (544/2388; 22.81%) vs. FSH/LH (589/2363; 24.93%) (P = 0.31; OR = 0.93, 95% CI = 0.81–1.07)] (Fig. 3).

The clinical pregnancy rate (CPR) significantly favored additional LH activity [recFSH (748/2758; 27.1%) vs. FSH/LH (838/2772; 30.2%) (P = 0.008; OR = 0.85, 95% CI = 0.76–0.96)], whereas the number of oocytes was higher with FSH-only protocols (recFSH vs. FSH/LH).

Figure 1

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>FSH only Events</th>
<th>FSH + LH activity</th>
<th>FSH only Events</th>
<th>FSH + LH activity</th>
<th>Total Events</th>
<th>Weight</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andersen 2006</td>
<td>82</td>
<td>368</td>
<td>96</td>
<td>363</td>
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<td>0.80 [0.57, 1.12]</td>
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<tr>
<td>Bosch 2008</td>
<td>44</td>
<td>140</td>
<td>48</td>
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<td>0.88 [0.53, 1.45]</td>
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<tr>
<td>Estesew 2007</td>
<td>76</td>
<td>193</td>
<td>63</td>
<td>193</td>
<td>1120</td>
<td>100.0%</td>
<td>1.34 [0.88, 2.03]</td>
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<tr>
<td>Fabregues 2006</td>
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<td>21</td>
<td>60</td>
<td>1120</td>
<td>100.0%</td>
<td>1.00 [0.47, 2.12]</td>
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<td>Klami 2003</td>
<td>11</td>
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<td>12</td>
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<td>100.0%</td>
<td>0.89 [0.35, 2.27]</td>
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<td>Rashidi 2005</td>
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<td>4</td>
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<td>1120</td>
<td>100.0%</td>
<td>0.72 [0.15, 3.54]</td>
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<tr>
<td>Strowitzki 2007</td>
<td>4</td>
<td>30</td>
<td>7</td>
<td>30</td>
<td>1120</td>
<td>100.0%</td>
<td>0.61 [0.13, 2.90]</td>
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<tr>
<td>Tarlatzi 2006</td>
<td>10</td>
<td>59</td>
<td>6</td>
<td>55</td>
<td>1120</td>
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<td>1.67 [0.56, 4.94]</td>
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<tr>
<td>Westergaard 2001</td>
<td>53</td>
<td>190</td>
<td>67</td>
<td>189</td>
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<td>0.70 [0.46, 1.09]</td>
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<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>1120</strong></td>
<td><strong>1110</strong></td>
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<td></td>
<td></td>
<td></td>
<td><strong>0.90 [0.75, 1.09]</strong></td>
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</tbody>
</table>

Heterogeneity: Tau² = 0.00; Chi² = 7.30, df = 8 (P = 0.50); P = 0%
Test for overall effect: Z = 1.05 (P = 0.29)

Live birth rate.

Figure 2

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>FSH only Events</th>
<th>FSH + LH activity</th>
<th>FSH only Events</th>
<th>FSH + LH activity</th>
<th>Total Events</th>
<th>Weight</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
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<tr>
<td>Andersen 2006</td>
<td>8</td>
<td>368</td>
<td>8</td>
<td>363</td>
<td>1888</td>
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<td>0.99 [0.37, 2.66]</td>
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<td>Balasch 2003</td>
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<td>30</td>
<td>0</td>
<td>30</td>
<td>1888</td>
<td>100.0%</td>
<td>Not estimable</td>
<td></td>
</tr>
<tr>
<td>Bosch 2008</td>
<td>2</td>
<td>140</td>
<td>2</td>
<td>140</td>
<td>1888</td>
<td>100.0%</td>
<td>1.00 [0.14, 7.20]</td>
<td></td>
</tr>
<tr>
<td>EIBO 2002</td>
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<td>386</td>
<td>7</td>
<td>395</td>
<td>1888</td>
<td>100.0%</td>
<td>0.58 [0.17, 2.00]</td>
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<td>Fabregues 2006</td>
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<td>60</td>
<td>0</td>
<td>60</td>
<td>1888</td>
<td>100.0%</td>
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<td>Hompes 2008</td>
<td>8</td>
<td>317</td>
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<td>312</td>
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<td>Jansen 2008</td>
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<td>54</td>
<td>0</td>
<td>54</td>
<td>1888</td>
<td>100.0%</td>
<td>Not estimable</td>
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<tr>
<td>Jansen 2002</td>
<td>4</td>
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<td>5</td>
<td>362</td>
<td>1888</td>
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<tr>
<td>Klami 2003</td>
<td>1</td>
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<td>1888</td>
<td>100.0%</td>
<td>0.32 [0.06, 3.18]</td>
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</tr>
<tr>
<td>Sauer 2004</td>
<td>1</td>
<td>21</td>
<td>1</td>
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<td>1888</td>
<td>100.0%</td>
<td>1.00 [0.06, 17.12]</td>
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</tr>
<tr>
<td>Strowitzki 2007</td>
<td>3</td>
<td>30</td>
<td>1</td>
<td>30</td>
<td>1888</td>
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<td>3.22 [0.32, 32.89]</td>
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<tr>
<td>Tarlatzi 2006</td>
<td>3</td>
<td>59</td>
<td>0</td>
<td>55</td>
<td>1888</td>
<td>100.0%</td>
<td>6.88 [0.35, 136.22]</td>
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<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>1888</strong></td>
<td><strong>1843</strong></td>
<td><strong>100.0%</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>1.08 [0.63, 1.83]</strong></td>
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</tbody>
</table>

Heterogeneity: Tau² = 0.00; Chi² = 7.50, df = 8 (P = 0.49); P = 0%
Test for overall effect: Z = 0.27 (P = 0.79)

Ovarian hyperstimulation rate.
Subgroup analysis of CPR showed a significant difference in favor of hMG, but not recFSH + recLH. Therefore, LH activity per se could not be the underlying mechanism accounting for this significant difference (Figs 4 and 5).

Discussion

LH is a glycoprotein that plays a crucial role in folliculogenesis during the natural ovarian cycles. It exerts its activity in theca cells, inducing androgen production, the substrate of estradiol, as well as in granulosa cells, where it regulates, together with FSH, the local production of various molecules (inhibin B and growth factors) that are important in promoting follicular maturation [7]. Despite this clear physiological role of LH in folliculogenesis, the role of exogenous LH activity in ovarian stimulation has been the subject of intense debate [8,9]. Premature luteinization and spontaneous ovulation resulting from premature LH surges presented significant challenges to early ovarian stimulation protocols until the introduction of GnRHa, which significantly reduced the incidence of LH surges and increased the pregnancy rates by overcoming this unwanted phenomenon [10]. That early researchers did not pay much attention to the low levels of LH resulting from this desensitization, as at that time, ovarian stimulation was carried out using gonadotrophins with LH activity. Nonetheless, with the introduction of gonadotrophins devoid of LH activity in the 1990s [11–13], the interest in the role of LH during ovarian stimulation started to grow.

The perplexity created by the contradictory data on the essential physiological role of LH and its debatable therapeutic role has further motivated researchers to assess the importance of LH activity during follicular development and its potential value during ovarian stimulation for IVF by comparing FSH-only protocols with LH-containing protocols in RCTs.

For FSH-only protocols, recFSH is the purest form of FSH that contains no LH activity, whereas LH activity can be provided as hMG (from urinary source) or as recombinant LH (recLH).

Comparing recFSH versus hMG (urinary source LH)

Earliest meta-analysis that compared recFSH and hMG treatment in normogonadotrophic patients undergoing a GnRHa long protocol was carried out by Van Wely et al. [14] that included four RCTs. The authors failed to show any statistically significant difference in the main outcome measures (ongoing pregnancy rates and live birth rate). As more RCTs addressing the same question were carried out, systematic reviews and meta-analyses were subsequently published summarizing the extracted data [3,4].

The study carried out by Coomarasamy et al. [3] included seven RCTs [15–20] (European and Israeli Study Group on highly purified menotropin vs. recombinant FSH, 2002) with a total of 2159 patients. In that meta-analysis, live birth rates were significantly increased by 4% in patients treated with hMG compared with those treated with recFSH (95% CI: 0.4–7.5).

A subsequent systematic review and meta-analysis by Al-Inany and colleagues included two more studies [21,22].
and a further 689 patients and confirmed the above finding [4] as live birth rates were found to be significantly increased by 3.4% (95% CI: 0.3–6.5) in women randomized to receive hMG compared with those randomized to receive recFSH after downregulation with a long GnRHa protocol.

The evaluation of the secondary outcome measures including the occurrence of OHSS and of multiple pregnancies did not show significant differences in both meta-analyses [3,4]. However, in the meta-analysis by Al-Inany and colleagues, the duration of ovarian stimulation was significantly shorter in women receiving hMG compared with those who received recFSH (weighted MD: –1.21 days, 95% CI: –1.35 to –1.06) after downregulation with a long GnRHa protocol.

Whether the GnRH antagonist protocol could yield similar or different results compared with the GnRH agonist long protocol was addressed in an RCT by Bosch et al. [23], who evaluated the IVF outcome in 280 patients stimulated with hMG (n = 140) vs. recFSH (n = 140). In this study, the number of oocytes retrieved was significantly reduced in patients treated with hMG compared with those treated with recFSH [(mean ± SD) 11.3 ± 6.0 vs. 14.4 ± 8.1, respectively; difference: –3.1 cumulus–oocyte complexes, 95% CI: –4.9 to –1.3]. However, the delivery rates were not significantly different between patients randomized to receive hMG vs. those randomized to receive recFSH (34.3 vs. 31.4%, respectively; risk difference: +2.9, 95% CI: –8.1 to +13.7) [23].

**recFSH versus recFSH + recLH**

Several researchers have investigated the impact of recLH addition during ovarian stimulation on the outcome of IVF/ICSI. A recent meta-analysis has summarized the data...
extracted from seven RCTs [24–31] including a total of 701 patients to determine whether the addition of recLH increases the live birth rate in IVF patients treated with FSH and GnRHa [32]. No statistically significant differences in the live birth rates were observed between patients who received recLH and those who did not when the seven eligible RCT were pooled (95% CI: –7.7 to +4.85, \( P = 0.65 \); heterogeneity \( P = 0.32 \), fixed-effects model). The result did not change in all subgroup analyses carried out on the basis of the type of GnRH analogue used for LH surge inhibition, the time or dose of recLH addition, or patient’s age.

Consistent with the above findings, the results of a large RCT carried out by Nyboeandersen and colleagues in which 526 women were randomized to be stimulated with either recFSH alone (n = 261) or recFSH with the addition of recLH (n = 265) from day 6 of stimulation onwards showed that the ongoing pregnancy rates at week 10–12 were not significantly different between the groups (28.7 vs. 27.2%, respectively; RD: +1.5%, 95% CI: –6.1 to +9.2).

**recFSH versus LH-containing protocols (either hMG or recFSH + rec LH)**

In our current meta-analysis, we aimed to evaluate the role of exogenous LH in ovarian stimulation irrespective of its source, that is, whether extracted from urine (in the form of hMG) or manufactured by recombinant DNA technology (recLH), and also irrespective of the mode of desensitization, that is, whether achieved by the long GnRHa protocol or by the rapidly acting GnRH antagonist protocol.

The results of this systematic review and meta-analysis failed to show any significant difference in the major outcome measures (live birth rate and OHSS) when comparing COH using FSH-only protocols with LH-containing protocols, suggesting that LH is not of paramount importance in ovarian stimulation. Moreover, LH-containing protocols showed a detrimental effect on the number of oocytes retrieved, which was higher with FSH-only protocols than with LH-containing protocols.

Although in the current review the CPR favored additional LH activity significantly, this can be questioned because the subgroup analysis of CPRs showed a significant difference in favor of hMG, but not in favor of recFSH + recLH. Therefore, LH activity per se may not be the underlying mechanism accounting for this significant difference.

In this respect, it is worth noting that hMG differs from recFSH not only in the LH activity content but also in the nature of the FSH contained. Indeed, the human-derived FSH contained in hMG carries a more extensive glycosylation and is closer in its isoform distribution to the pituitary FSH. In contrast, recFSH carries a rodent-type glycosylation that is simpler in structure and that includes a lower number of sialic acid residues. The glycosylation and sialilation status of FSH affects its half-life [33] and a variety of FSH biological properties [34]. It is therefore possible that some of the differences found between hMG and recFSH are actually because of the different nature of the FSH component. Our findings that demonstrated better pregnancy rates in hMG protocols and not in recFSH + recLH protocols further endorse this interpretation. Accordingly, possible differences in the FSH component should be taken into due account in future works investigating the value of the LH supplementation.

**FSH isoforms: a new theory for the endocrine aspect of follicular and oocyte growth**

The clinical efficacy of commercially available gonadotrophin preparations has been the subject of an intense debate primarily focused on the origin of FSH activity (urine vs. recombinant derived) and whether the preparation included LH like activity. FSH isoform composition has received little or no attention, and is usually considered to
have a negligible effect on clinical effectiveness. However, in a study carried out by Andersen et al. [35], it was reported that the FSH isoform profile of commercial gonadotrophin preparations is of clinical importance and should be taken into account when evaluating endocrine aspects and efficacy for each preparation.

FSH exists as a family of isoforms with distinct oligosaccharide structures, and the FSH isoform mixtures released change during the follicular phase of the menstrual cycle. The different isoforms exert a number of different and divergent biological effects. Exposure of cumulus-oocyte complexes to less-acidic FSH isoforms in a pulse-like manner results in a rapid pattern of cAMP accumulation exceeding that seen with acidic isoforms. It appears that pulsatile and intermittent release of less-acidic/short-living FSH isoforms is sufficient to induce biological responses while allowing the granulosa cell FSH receptors to regain responsiveness to further FSH stimulation. This may explain the elevation of progesterone in recombinant forms (less-acidic isoforms) as these isoforms are more potent than their counterparts of urinary origin. Future studies should evaluate the difference in the premature increase in progesterone in recFSH and urinary FSH cycles [36].

We can firmly conclude from the current available evidence at hand that the addition of LH activity to FSH in ovarian stimulation irrespective of its source, that is, whether urinary or recombinant, and irrespective of the pituitary desensitization protocol, that is, whether a GnRHa long protocol or a GnRH antagonist, did not show any superiority over FSH-only protocols in terms of the live birth rate, OHSS rate, and ongoing pregnancy rates. However, additional LH activity provided as hMG showed higher CPRs, although it did not when provided as recLH, indicating that factors other than LH activity per se may be responsible for this significant difference. FSH-only protocols yielded more number of retrieved oocytes than LH-containing protocols.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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A comparative study between cabergoline, coasting, and step-down regimens in the prevention of severe OHSS and their correlation with pregnancy rate in intracytoplasmic sperm injection cycles

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Objective
To compare coasting, cabergoline, and step-down methods in the prevention of severe ovarian hyperstimulation syndrome (OHSS) and their effects on various outcome measures in intracytoplasmic sperm injection cycles.

Materials and methods
Patients at risk of developing OHSS were randomized into three groups: group A, which included 16 patients who had undergone coasting by withholding human menopausal gonadotropin while continuing the gonadotropin-releasing hormone analogue till the E2 decreased below 3000 pg/ml before triggering, group B (cabergoline group), which included 28 patients who received 0.5 mg/day for 8 days starting from the day of human chorionic gonadotropin, and group C (step-down group), which included 30 patients who had decreased the dose of human menopausal gonadotropin by 1 ampoule to decrease E2 below 3000 pg/ml then triggering by 10 000 IU of human chorionic gonadotropin and oocytes retrieved after 34–36 h later.

Results
The number of oocytes retrieved, M2, and number of fertilized oocytes were higher in the cabergoline group than the other two groups \( (P<0.05) \), but the clinical pregnancy rate was higher in the coasting group (50%) compared with the other two groups and the lowest in the cabergoline group (14.3%), with no statistical significance between the three groups in the development of severe OHSS \( (P=0.41) \).

Conclusion
Coasting may have a higher pregnancy rate and may represent a higher preventive method for the development of severe OHSS than the other two methods.

Keywords: cabergoline, coasting, intracytoplasmic sperm injection, ovarian hyperstimulation syndrome, step-down

Introduction
Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of ovarian stimulation for assisted reproduction technology and other infertility treatments; usually, it develops several days after oocytes are retrieved or after assisted ovulation. This syndrome is characterized by ovarian enlargement because of multiple ovarian cysts and acute fluid shift into the extracellular space including ascites, hemococoncentration, hypovolemia, and electrolyte imbalance [1].

Grades of OHSS are mild, including grade I, II, moderate grade III, and severe OHSS grade IV, V [2]. Its pathogenesis is related to increased vascular permeability in the region surrounding the ovaries and their vasculature [3]. 

\( \beta \)-Human chorionic gonadotropin (HCG) and its analogs have been implicated in the past. Vasoactive substances such as interleukins, tumor necrosis factor-\( \alpha \) endothelial, and vascular endothelial growth factor (VEGF) secreted by the ovaries have been implicated in the increase in vascular permeability [4].

VEGF not only stimulates new blood vessel development in the ovary but also induces vascular hyper permeability by interacting with the VEGF receptor [5]. The rates of OHSS are as follows: mild, 8–23%, moderate, 1–7%, and severe, 0.25–5% [6]. Once the syndrome occurs, little can be done to alter the course of events and only supportive measures can be carried out; however, a definite and useful method is cycle cancelation or no HCG administration. Many other methods have been used to prevent the syndrome. The appropriate protocol and gonadotropin needs to be chosen [7]. Unilateral ovarian follicular aspiration before the administration of HCG was successful in some investigations [8]. In another study, aspiration of half of the follicles before the administration of HCG reduced the risk of severe OHSS [9].
Another method for the prevention of OHSS is the coasting approach withholding gonadotropin administration, which has been used in ovulation induction cycles to prevent excessive response [10]. Recently, a dopamine-agonist, cabergoline, has been used successfully for the prevention of severe OHSS (anti-VEGF) [11]. The step-down human menopausal gonadotropin (HMG) protocol seems rational as it mimics the natural ovarian cycles when ultrasound monitoring (usually by stimulation day 7) reveals the advancement of a reasonable number of developing follicles [12]. The impact of this kind of management of patients with risk factors is that earlier close monitoring of serum E2 and ultrasound could be valuable in determining early evolution of OHSS [13]. Approach might be viewed as further development of conventional ‘coasting’, yet avoiding the risk of abrupt E2 fall with reduction of oocyte retrieval rate and embryo quality [14].

The aim of this study was to determine the effect of cabergoline on the cycle outcome and prevent severe OHSS and to compare it with other popular methods such as the coasting method and the step-down method (decreasing the dose of HMG) in preventing severe OHSS and in the detection of the cycle outcome and pregnancy rate.

**Patient and methods**

The study was approved by the ethical committee of research in Kasr El-Aini Hospital, Cairo University. This was a prospective cohort study of couples who were subjected to intracytoplasmic sperm injection cycles in the in-vitro fertilization (IVF) unit in Kasr El-Aini Hospital, Cairo University. Seventy-four women at risk of developing OHSS were included in the study. This included young women below 35 years of age, PCO patients, those with E2 more than 4000 pg/ml during the follow-up of induction, women with a necklace sign (antral follicle count >20) before induction, and women with preovulatory follicular number more than 20 follicles on both sides or a large number of follicles more than 15 mm during the course of induction.

All women were undergoing the long protocol for stimulation of cycle. They were downregulated with a gonadotropin-releasing hormone analogue (GnRH-a) subcutaneously (Decapeptyl 0.1 mg daily from day 20 in the previous cycle of stimulation). Then, daily administration of HMG was carried out intramuscularly along with the (GnRH-a). E2 was administered for women at risk for OHSS to determine the level and counsel the patient to complete the cycle or not. Women who were at risk of developing OHSS and did not want to cancel their cycle were divided into three groups.

Group A, the coasting group, included 16 women, for whom we continued the GnRH-a, but withheld the gonadotropins until serum E2 started to decrease, with the mean duration of coasting being 2–3 days. Then HCG was administered to this group of women when E2 was below 3000 pg/ml.

Group B, the cabergoline group, included 28 women who received 0.5 mg/day for 8 days starting from the day of HCG administration. Group C, the step-down group, included 30 women in whom the dose of gonadotropins was decreased by 1 ampoule until the E2 started to decrease. In all three groups, when at least three follicles of at least 18 mm in size were achieved, the HCG dose (10000 IU) was administered for triggering.

After the administration of HCG, all three groups were followed up every 48 h for the detection of severe OHSS; Hb, hematocrit value, and kidney functions were determined by follow-up ultrasound for all patients. The embryo transfer procedure was carried out for all women using the same method and luteal support was carried out for all women using progesterone in oil (800 mg into two divided doses) for 14 days. Clinical pregnancy was detected by the presence of a gestational sac or cardiac pulsation 3 weeks after ET.

Data were statistically described in terms of the mean (SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was carried out using the Kruskal–Wallis test, with the Mann–Whitney U-test for independent samples carried out for post-hoc multiple two-group comparisons. For comparison of categorical data, the χ²-test was carried out. The exact test was used when the expected frequency was less than 5. P-values less than 0.05 were considered statistically significant. All statistical calculations were carried out using computer programs statistical package for the social science (SPSS Inc., Chicago, Illinois, USA) version 15 for Microsoft Windows.

**Results**

The study was carried out between March 2010 and August 2011; we studied 74 patients at the IVF center in Kasr El-Aini Hospital, Cairo University. The patients were divided into three groups: 28 patients in the cabergoline group, 30 patients in the step-down group, and 16 patients in the coasting group, and were evaluated. The mean age of the patients was 27.4 ± 6.0 years in the coasting group, 29.4 ± 3.7 years in the cabergoline group, and 30.1 ± 5.1 years in the step-down group (P = 0.122).

The mean duration of infertility was 5.41 ± 3.7 years in the coasting group, 6.0 ± 4.18 years in the cabergoline group, and 5.2 ± 5.05 years in the step-down group (P = 0.762). The mean BMI was 27.9 ± 3.1 in the coasting group, 32.6 ± 6.0 in the cabergoline group, and in 32.8 ± 3.7 the step-down group (P = 0.001).

Antral follicle count was 17.88 ± 2.14 in the coasting group, 17.84 ± 2.34 in the cabergoline group, and 18.2 ± 2.18 in the step-down group (P = 0.806). The number of eight-cell embryos was 1.06 ± 2.331 in the coasting group, 1.07 ± 1.783 in the cabergoline group, and 2.05 ± 3.349 (P = 0.005) in the step-down group. The mean number of retrieved oocytes was 14.41 ± 5.173 in the coasting group and 21.50 ± 8.307 in the cabergoline
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Table 1 Patient demographic data

<table>
<thead>
<tr>
<th>Outcome measures</th>
<th>Coasting</th>
<th>Cabergoline</th>
<th>Step-down</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.4 ± 6</td>
<td>29.4 ± 3.7</td>
<td>30.1 ± 5.1</td>
<td>0.122</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>6.4 ± 3.7</td>
<td>6.2 ± 4.18</td>
<td>5.2 ± 5.06</td>
<td>0.782</td>
</tr>
<tr>
<td>BMI</td>
<td>27.9 ± 3.1</td>
<td>32.6 ± 6.0</td>
<td>32.8 ± 3.27</td>
<td>0.001</td>
</tr>
<tr>
<td>AFC</td>
<td>17.88 ± 2.14</td>
<td>17.84 ± 2.34</td>
<td>18.2 ± 2.18</td>
<td>0.806</td>
</tr>
<tr>
<td>E2 of day HCG</td>
<td>4512 ± 752</td>
<td>4398 ± 1906</td>
<td>4120 ± 2978</td>
<td>0.156</td>
</tr>
</tbody>
</table>

| Number of retrieved oocytes          | 14.41 ± 5.17 | 21.5 ± 8.307| 15.73 ± 6.26| 0.001      |
| Number of MII                        | 73 ± 129     | 13.86 ± 5.536| 11.27 ± 4.763| 0.001      |
| Number of fertilized oocytes         | 5.86 ± 2.93  | 10.07 ± 4.22 | 8.47 ± 5.366| 0.002      |
| Number of eight-cell embryos         | 1.06 ± 2.33  | 1.07 ± 1.78  | 3.53 ± 4.34 | 0.005      |
| Chemical pregnancy rate              | 8 (50%)      | 4 (14.3%)    | 10 (33.3%)  | 0.038      |
| Rate of OHSS                          | 0            | 2            | 1          | 0.481      |

AFC, antral follicle count; HCG, human chorionic gonadotropin; OHSS, ovarian hyperstimulation syndrome.

Discussion

One of the major, dangerous complications of ovarian stimulation in IVF-intracytoplasmic sperm injection women is OHSS, which may be life threatening. There are some measures for the prevention of OHSS; the most effective method of prevention is cancelation of the cycle, but in most of the IVF centers, the couple refuses the cancelation because of financial and emotional causes. Another method for the prevention of OHSS is freezing all embryos and postponing embryo transfer to the subsequent cycle [15]; also, another approach for the prevention of OHSS is follicular aspiration before the administration of HCG [8]. There are other methods for reducing the risk for severe OHSS; one of these methods is coasting, which is withholding the administration of gonadotropin and postponing the HCG injection while continuing the GnRH antagonist till the E2 level increases to less than 3000 pg/ml. Coasting causes atresia of a large number of small follicles, and thus leads to a decrease in the serum E2 level and vasoactive mediators [16]. A decrease in the serum level of FSH concentrations downregulates the LH receptors of the follicles; thus, this results in the maturation of a fewer number of oocytes by HCG and leads to a reduction in the number of oocytes retrieved [16].

In our study, we used cabergoline for the prevention of severe OHSS, which has been used successfully in high-risk patients for OHSS. Cabergoline inactivates the VEGFR-2; this growth factor is very important in endometrial angiogenesis during the beginning of the secretory phase [17]. Moreover, VEGF expression is important in the growth and development of the endometrium with advancing stages of the menstrual cycle. VEGF expression has been especially correlated with implantation of the embryo [17]. It has been shown that pharmacological doses of cabergoline can inhibit VEGF-mediated microvascular permeability, proliferation, and migration of endothelial cells in vitro by inducing endocytosis of VEGF-R2 [17]. Another study has shown that changes in VEGF-R2 induced by low-dose cabergoline reversed the occurrence of increased vascular permeability without altering angiogenesis [18]. Cabergoline used for the prevention of OHSS reduced neither the rate of pregnancy nor the implantation rate [18].

In our study, we found that the number of retrieved oocytes in the cabergoline group was higher than that of the coasting group \((P = 0.003)\); also, the number of MII and number of fertilized oocytes in the cabergoline group

group (the \(P\)-value between these two groups was 0.003), with a statistically significant difference. The mean number of retrieved oocytes in the step-down group was 15.73 ± 6.264 (\(P\)-value between the step-down and the coasting group was 0.563), with no statistical significance. The \(P\)-value between the cabergoline and the step-down group was 0.002, with the overall \(P\)-value equal to 0.001.

The mean number of MII was 7.50 ± 2.129 in the coasting group and 13.86 ± 5.536 in the cabergoline group, with a statistical significance between the coasting and the cabergoline group \((P = 0.001)\), and the mean number of MII in the step-down group was 11.27 ± 4.763, with no statistical significance between the coasting and the step-down group \((P = 0.005)\), and also no statistical significance between the cabergoline and the step-down group \((P = 0.130)\); the overall \(P\)-value is equal to 0.001.

The mean number of fertilized oocytes was 5.65 ± 2.936 in the coasting group and 5.00 ± 4.225 in the cabergoline group \((P = 0.001)\). The mean number of fertilized oocytes was 9.47 ± 5.355 in the step-down group \((P = 0.007)\) between the coasting and the step-down group, \(P = 0.4115\) between the cabergoline and the step-down group, and overall \(P = 0.002\). The chemical pregnancy rate was eight women (50%) in the coasting group, four women (14.3%) in the cabergoline group, and 10 women (33.3%) in the step-down group; the overall \(P\)-value was 0.038.

The number of women who developed severe OHSS was zero in the coasting group, two women in the cabergoline group, and one woman in the step-down group; the overall \(P\)-value was 0.481. The mean E2 on the day of HCG was 4512 ± 752 in the coasting group, 4396 ± 1906 in the cabergoline group, with no statistical significance between them \((P = 0.687)\), and 4120 ± 2978 in the step-down group, with no statistical significance between the coasting and the step-down groups \((P = 0.004)\) and also no statistical significance between the cabergoline and the step-down groups \((P = 0.171)\); the overall \(P\)-value was 0.156 (Table 1).
were higher than those of the coasting group ($P = 0.001$ and 0.002, respectively). The eight-cell embryos in the coasting group (1.06 ± 2.331) were less than those in the cabergoline group (1.07 ± 1.783) ($P = 0.511$).

Also, the chemical pregnancy rate in the coasting group (50%) was higher than that of the cabergoline group (14.3%) ($P = 0.016$); although the number of oocytes retrieved was affected by coating, the endometrial receptivity was affected in the cabergoline group and thus the clinical pregnancy rate was lower in the cabergoline group.

These results are in agreement with those of Aflatoonian et al. [19], as they found that the number of retrieved oocytes, number of MII, eight-cell embryos, and the number of patients who developed severe OHSS is higher in the cabergoline group than in the coasting group. However, our results differ from theirs in terms of the pregnancy rate as they found that the pregnancy rate in the cabergoline group was higher than that of the coasting group.

In terms of the development of severe OHSS in the coasting group, no cases were reported, but in the cabergoline group, two women developed severe OHSS, with no statistical significance between them ($P = 0.48$).

The third method we used in this study was the step-down of the HMG by 1 ampoule in suspicious cases for OHSS and follow-up by E2 every other day to reach less than 3000 pg/ml to yield HCG. The step-down in induction of ovulation will allow more follicles to undergo atresia, thus reducing the overall number of follicles capable of secretory activity by the time of HCG administration; thus, this leads to a reduction in the rate of OHSS [20]. On comparison of the coasting and the step-down group, we found that the number of retrieved oocytes in the step-down group was higher than that of the coasting group, but with no statistical significance between them ($P = 0.563$), and the number of MII in the step-down group was higher than that of the coasting group ($P = 0.005$) (positive statistical significance). Also, the number of fertilized oocytes in the step-down group was higher than that of the coasting group, with no statistical significance ($P = 0.007$). The number of eight-cell embryos in the step-down group was higher than that of the coasting group, with no statistical significance ($P = 0.009$).

Also, in terms of the eight-cell embryo number in each group, there was a significant difference among the three groups, with an overall $P$-value equal to 0.005.

The chemical pregnancy rate in the step-down group was 33.3% (10 cases), which is lower than the chemical pregnancy rate in the coasting group of 50% (eight cases) ($P = 0.270$). In the step-down group, only one woman developed severe OHSS, but in coasting group, none of the women developed severe OHSS, with no statistical significance between them.

When we compared the results of the step-down group and the cabergoline group, it was found that the number of retrieved oocytes in the step-down group was lower than that of the cabergoline group ($P = 0.002$), but with no statistical significance between both groups. The number of MII and number of fertilized oocytes ($P = 0.130$ and 0.415, respectively) in the step-down group were lower than those of the cabergoline group. The chemical pregnancy rate was 33.3% (10 cases) in the step-down group and 14.3% (four cases) in the cabergoline group ($P = 0.090$). There was no statistical significance in the occurrence of severe OHSS among both groups.

**Conclusion**

We found that the number of retrieved oocytes, MII, and fertilized oocytes were higher in the cabergoline group than the other two groups, but the chemical pregnancy rate in the coasting group was higher than that of the other two groups, and the cabergoline group had the lowest pregnancy rate, with no difference in the development of severe OHSS.

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**Conflicts of interest**

There are no conflicts of interest.

**References**


Antral follicular count is a better indicator for the assessment of ovarian reserve
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Introduction

Ovarian reserve (OR), a term that has evolved in the era of assisted reproductive technology (ART), refers to the residual oocyte–granulosa cell repertoire that, at any given age, is available for procreation. Both quantitative and qualitative deteriorations in the oocyte complement, and therefore a waning OR, are recognized phenomena associated with advancing age. Although a decline in OR thus accompanies chronological aging, there is an acceleration in this process [1].

Diminished OR is defined as a decrease in the reproductive potential of a woman’s remaining oocytes to the point at which the probability of producing a viable gestation is low. The age-related decline in reproductive potential is the principal factor contributing toward the increase in the prevalence of infertility in western society [2]. A cut-off level of less than six antral follicles used by Kwak et al. [3] had a sensitivity of 41% and a specificity of 95%, with a prevalence of 27% for a poor response. In the population studied after ovarian hyperstimulation in an IVF treatment, the accuracy was 89% [3].

OR tests can identify the high-risk population of poor response, and hence yield information that affects prognosis, counseling, and treatment decisions. Abnormal tests in older women can help to persuade patients to abandon plans to pursue aggressive and costly treatment. On the other hand, mild abnormal test in young women can help to convince them to do just the opposite and to take the fullest advantage of a rapidly closing window of opportunity [4].

Over the past two decades, a number of so-called ovarian reserve tests (ORTs) have been designed to determine oocyte reserve and quality and have been evaluated for their ability to predict the outcome of IVF in terms of oocyte yield and the occurrence of pregnancy. Many of
these tests have become part of the routine diagnostic procedure for infertility patients who undergo assisted reproductive techniques. The unifying goals are traditionally to determine how a patient will respond to stimulation and their chances of pregnancy [5].

Several tests have been proposed to define the OR status of individual patients and predict fertility outcomes for assisted reproduction and these include age, history of canceled cycles, clinical symptoms (e.g. oligomenorrhea), basal blood tests [follicle-stimulating hormone (FSH)], luteinizing hormone (LH), anti-Mullerian hormone (AMH), estradiol (E2), FSH/LH ratio, inhibin B, progesterone (P4), P4:E2 ratio, testosterone, vascular endothelial growth factor (VEGF), insulin-like growth factor-1 : insulin-like growth factor binding protein-1 ratios (IGF-1 : IGFBP-1 ratio)].

Materials and methods

Patients

This is a prospective cross-sectional study carried out at the IVF center of the Egypt International Hospital between January 2007 and February 2009, and included 86 patients who would be undergoing IVF or ICSI for the first time with the following inclusion criteria: age from 31 to 49 years, cause of infertility male factor, tubal factor, or unexplained infertility, basal FSH level below 15 mIU/ml, both ovaries well visualized by transvaginal sonography, no history of ovarian surgery, no history of endocrinological disorder, and no history, symptoms, or signs of endometriosis. The study was discussed with the patient before her participation and consent was taken.

Study protocol

All patients of the study were recruited on days 2–5 of the cycle; before the ovulation protocol was carried out, they were subjected to the following: full history taking and a thorough clinical examination, transvaginal sonography for the assessment of the AFC, both ovaries, and the uterus, and to exclude any pelvic pathology. For ultrasonographic evaluation, we used transvaginal 7.5 MHz (Sonoace 9900; Medison, Seoul, Korea).

Measurements of basal serum FSH, E2, progesterone, and AMH were carried out. Also, routine laboratory tests such as complete blood test and assessment of liver and kidney functions were carried out.

Induction of ovulation was carried out using the long luteal phase protocol. All patients received Decapeptyl (GnRHa) 0.1 µg subcutaneous injection daily at 8 p.m. starting from day 20 of the cycle and for 2 weeks, after ensuring downregulation by serum E2 level; human menopausal gonadotropins (hMG) injection was administered at a starting dose of 150–300 IU/day for 7 days and then adjusted according to the response. The patient was monitored every other day for folliculometry. Human chorionic gonadotropin (hCG) was administered if the mean follicular diameter was 18–20 mm at a dose of 10,000 IU intramuscular injection; if the mean diameter of the follicles was less than 10 mm after 14 days, the cycle was canceled. Ovum pick up was carried out after 36 h of hCG and embryo transfer was carried out on day 2 or 3. Pregnancy was confirmed by a positive qualitative B-hCG test 2 weeks after embryo transfer.

Hormone assays

Serum FSH, E2, and AMH were measured on cycle day 3, whereas progesterone was measured on cycle day 1. Both E2 and progesterone were measured again on the day of hCG administration. Serum FSH was measured using radioimmunoassay (RIA) (Gambyt-CR; Diagnostic Products Corporation, Los Angeles, California, USA). The interassay coefficient of variation (CV) of FSH was 3.7%. The intra-assay CV was 4.7%.

Serum AMH was measured using two enzyme-linked immunosorbent assays. In the traditional assay, Immulon two plates (Dynatech Corp., Chantilly, Virginia, USA) were coated with monoclonal antibody 10.6 (Dr R. Cate, Cambridge, Massachusetts, USA), raised against recombinant human AMH by an overnight incubation at room temperature. Sera were assayed at 1 : 4, 1 : 8, 1 : 16, and 1 : 32 dilutions in PBS containing 1% BSA. As the second antibody, we used polyclonal antibody L40, consisting of an immunoglobulin G fraction isolated by affinity chromatography on protein A-Sepharose from the serum of a rabbit immunized with recombinant human AMH. L40 was added to the wells at 1 µg/ml in PBS–1% BSA for 1 h at room temperature. Subsequently, an alkaline phosphatase-labeled goat anti-rabbit immunoglobulin G antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, Pennsylvania, USA) was added and incubated for a further hour before the reaction was visualized with an MRX spectrophotometer (Dynatech Corp.) at 405 nm, using paranitrophenyl phosphate (Sigma Chimie, Saint-Quentin-Fallavier, France) as a substrate.

 Estradiol levels were measured by RIA. Duplicate aliquots (0.2 ml) of plasma were fixed with 0.1 ml of a 1.5 mol/l sodium carbonate solution, pH 10.5, and extracted with 10 vol of diethyl ether. The mixture was frozen, the ether was decanted and evaporated, and the residue was dissolved in PBS and incubated with tritium-labeled E2 (Amersham International, Little Chalfont, Buckinghamshire, UK) and sheep anti-E2 antisera (Bioclin Services, Cardiff, Wales, UK). Bound and free E2 were separated using dextran-coated charcoal. The interassay CVs for two control plasmas were 9.5 and 6.6% for E2 concentrations of 174 and 409 pmol/l, respectively. Serum progesterone was measured using RIA (Gambyt-CR; Diagnostic Products Corporation). The intra-assay and interassay CV were 9.6 and 4.9%.

Outcome measures

The main outcome of the study was the ovarian response and was determined according to the number of oocytes retrieved; five or more was considered to indicate a good response and less than five was considered a poor
response. The secondary outcome was the chemical pregnancy rate (PR).

**Statistical analysis**

Data were statistically described in terms of range, mean ± SD, median, frequencies (number of cases), and relative frequencies (percentages) when appropriate. Comparison of quantitative variables between different groups in the present study was carried out using the Mann–Whitney U-test for independent samples. For comparison of categorical data, the χ² test was carried out. Exact test was used when the expected frequency was less than 5. Accuracy was represented using the terms sensitivity and specificity. Receiver-operator characteristic (ROC) analysis was used to determine the optimum cut-off value for the diagnostic markers studied. Correlations between various variables were determined using Pearson’s moment correlation and Spearman’s rank correlation equations. A probability value (P value) less than 0.05 was considered statistically significant. All statistical calculations were carried out using computer programs Microsoft Excel version 7 (Microsoft Corporation, New York, USA), SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, Illinois, USA), and the ArcusQuickStat (Biomedical version; Addison Wesley Longman Ltd, USA) statistical program.

**Results**

All the 86 patients were eligible for analysis; the patients were categorized according to the response into good responders, who retrieved five or more oocytes, or poor responders, who retrieved less than five, and according to the result of the B-hCG test as a positive test (chemical pregnancy) or a negative test. 81.4% (70 patients) were good responders and 18.6% (16 patients) were poor responders, and the chemical PR was 33.72% (29 patients).

Table 1 shows a comparison of both groups in terms of the general variables and it indicates that there was a statistically significant difference between both groups in terms of the patient age, duration of infertility, and number of hMG ampoules used. As the poor responders were older than the good responders and they had more long-standing infertility, they required more hMG ampoules to be stimulated.

In terms of the other variables, when we compared both groups, it was found that there was a statistically significant difference in the basal serum FSH level (P value 0.000), serum AMH (P value 0.001), and basal AFC (P value 0.003), whereas there was no statistically significant difference between both groups in the basal levels of E2 and progesterone. Measurement of the E2 and progesterone levels on the day of hCG administration indicated that there was a statistically significant difference between both groups in the E2 level, whereas the progesterone level was insignificant. Table 2 shows a comparison between both groups in terms of these variables.

On analyzing the previous data on the ROC curve characteristic, we found that basal AFC was superior to basal serum FSH and serum AMH in the prediction of the ovarian response; the best cut-off value, sensitivity, specificity, and likelihood ratio for each test are presented in Table 3.

The secondary outcome was the chemical PR, which was 33.72% (29 patients). Table 4 presents a comparison of the group with a positive pregnancy test and those with a negative pregnancy test in terms of the basal serum FSH,

<table>
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<th>Table 1 Background characteristics of the study population</th>
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<td>Duration of infertility (years)</td>
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<td>Mean number of hMG ampoules used</td>
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hMG, human menopausal gonadotropin; S, significant.

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<th>Table 2 Hormonal assay of follicle-stimulating hormone, Estradiol, progesterone, anti-Mullerian hormone</th>
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<td>Variables</td>
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<td>Basal serum AMH (ng/ml)</td>
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AFC, antral follicular count; AMH, anti-Mullerian hormone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; S, significant.
Discussion

In this study, we used different tests for the prediction of the ovarian response in 86 patients undergoing IVF or ICSI for the first time; we found that age showed a strong relationship with the response as the mean age for the good responders and the poor responders was (68 ± 4.391 and 38.44 ± 4.77), respectively. In a study carried out by Van Voorhis [8], it was concluded that age is a well-established predictor for the OR. And the good Odds Ratio can be suggested by age less than 38 years at the time of treatment, as the decay rate of resting follicles accelerates from 38 years onwards [9,10].

Also, a relatively longer duration of infertility was observed in poor responders, 10.27 ± 5.7 years, whereas in good responders, it was 5.43 ± 3.7 years. This can be attributed to the fact that patients with a poor OR would have undergone more trials of ICSI with poor results, thus increasing the duration of infertility. As reported by Hammadieh et al. [11], increasing duration of infertility is associated with a decrease in the probability of spontaneous pregnancy in untreated infertile couples (15.3% for 1–3 years, 14% for 4–6 years, 12.9% for 7–9 years, 12.4% for 10–12 years, and 8.6% for more than 12 years).

The number of hMG ampoules used in both good and poor responders showed a statistically significant difference (38.69 in good responders and 49.56 in poor responders), and this can be considered a logical result in poor responders. A small number of randomized-controlled trials and retrospective studies have evaluated the effectiveness of high-dose FSH regimens beyond...
The total number of follicles that can be stimulated under ovarian stimulation with FSH. ORTs are considered to indirectly reflect the size of the cohort of small antral follicles (2–10 mm in diameter) in the ovary. Thus, a decrease in follicle number increases the risk of a poor response after ovarian hyperstimulation in patients with IVF at an older age [3,14].

In our study, AFC was obviously lower in poor responders, 5.71, whereas it was 11.76 in good responders, with a statistically significant difference ($P$ value 0.003) (the mean AFC in all patients was 10.94). Taking $P$ value less than 0.05, AFC was considered significant for a poor response. In ROC curve analysis, area under the curve is greater than 75% (85.56), that is the value of testing AFC in predicting a response is high. With an optimum cut-off point = 8, the highest sensitivity and specificity that could be obtained were 85.71 and 73.46%, respectively. The likelihood ratio was 3.22. The mean value of AFC on cycle day 3 in pregnant patients was 8 ranging from 5 to 30. In nonpregnant patients, the mean value was 8.77, ranging from 3 to 17. There was a statistically significant difference ($P$ value 0.002). Area under the curve was less than 75% (70.45), that is the value of testing AFC in predicting pregnancy was low. At a cut-off value of 9.048, the sensitivity was 91.3% and specificity was 59.1%. The likelihood ratio was 2.23. And this agrees with that more number of decreases implantation rates [15]. Another study showed that a low AFC less than 10 antral follicles with a diameter measuring 2–10 mm was found to indicate reduced OR and decreased chance for pregnancy after ART [16].

The mean basal FSH among patients was 8 IU/ml. In good responders, it was $6.45 \pm 2.27$ IU/ml, whereas in poor responders it was $12.95 \pm 6.69$ IU/ml, with a statistically significant difference when considering $P$ value less than 0.05 as significant. In ROC curve analysis, area under the curve is greater than 75% (74.26), that is the value of testing FSH in predicting response is low. With an optimum cut-off point = 9.26 IU/ml, the highest sensitivity and specificity that could be obtained were 70 and 91.2%, respectively. The likelihood ratio was 7.927. The mean value of basal serum FSH in pregnant patients was $6.65 \pm 2.31$ IU/ml, ranging from 2.31 to 11.64 IU/ml. In nonpregnant patients, the mean value was $9.23 \pm 5.23$ IU/ml, ranging from 5.23 to 15 IU/ml. There was a statistically significant difference ($P$ value 0.001). In ROC curve analysis, area under the curve was less than 75% (64.85), that is the value of testing FSH in predicting pregnancy is low. With an optimum cut-off point = 4.94 IU/ml, the highest sensitivity and specificity that could be obtained were 33.33 and 95.46%, respectively. The likelihood ratio was 7.32.

The literature has consistently suggested that FSH testing is the best marker for assessing OR and for predicting the response to super ovulation, with a good correlation with PRs [17,18] and had significant correlations with FSH-hMG administered, total number of oocytes retrieved, conception rate, and the possibility of developing ovarian hyperstimulation syndrome [19,20]. A cut-off point of 5.25 mIU/ml for day 3 FSH was suggested for predicting OR [20].

Figure 2

Receiver-operator characteristic plot for antral follicle count in the prediction of a poor response.

Figure 3

Receiver-operator characteristic plot for basal anti-Mullerian hormone level in the prediction of a poor response.

maximal ovarian stimulation with FSH. ORTs are considered to indirectly reflect the size of the cohort of small antral follicles (2–10 mm in diameter) in the ovary. Thus, a decrease in follicle number increases the risk of a poor response after ovarian hyperstimulation in patients with IVF at an older age [3,14].

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However, an elevated FSH value does not necessarily mean that a woman will be unable to conceive a pregnancy naturally. There are many examples of patients with elevated FSH values who were subsequently able to conceive without treatment. The ORTs are better used as a predictor of patients who are not likely to benefit from ART; young women even with elevated FSH may still have a favorable IVF outcome [8].

The mean value of serum estradiol days 1–3 was 26.1 pg/ml in all patients, 26.67 pg/ml in good responders, 23.75 pg/ml in poor responders, with a statistically insignificant difference. The mean value of basal serum estradiol days 1–3 in pregnant patients was 18.94 pg/ml, ranging from 5 to 47 pg/ml. In nonpregnant patients, the mean value was 34.06 pg/ml, ranging from 10 to 189 pg/ml. There was a statistically insignificant difference (P value 0.177). In ROC curve analysis for the ability of basal day 3 serum estradiol to predict pregnancy, the area under the curve was less than 75% (54.35), that is the value of testing estradiol in predicting pregnancy is low. At a cut-off value of 47.32 IU/ml, the sensitivity was 100% and specificity was 15.38%. The likelihood ratio was 1.18.

In a study carried out to test the hypothesis that elevated E2 levels on day 3 of IVF cycles without a GnRH agonist are associated with reduced oocyte numbers and PRs, the ongoing PR per retrieval for patients with E2 levels less than 30 pg/ml was significantly higher than that for patients with E2 levels 31–75 pg/ml. There were no pregnancies if the E2 level was greater than 75 pg/ml. The mean number of oocytes per retrieval was significantly lower in patients from the E2 groups with E2 greater than 60 pg/ml compared with patients in groups with E2 less than 60 pg/ml. Day 3 FSH and E2 levels were also evaluated simultaneously. In patients with the lowest levels of FSH and E2, the PR was the highest. No pregnancies occurred if the FSH level was greater than 17 mIU/ml (conversion factor to SI unit, 1.00) and the E2 level was greater than 45 pg/ml on day 3 [21].

A systematic analysis of all studies testing basal serum estradiol as an ORT concluded that the clinical applicability for basal estradiol as a test before starting IVF is prevented by a very low predictive accuracy, both for poor response and for nonpregnancy [5].

The mean value of serum estradiol day hCG injection was 2239.43 pg/ml in all patients; 2580 pg/ml in good responders and 874.2 pg/ml in poor responders, with a significant statistical difference. The mean value of the basal serum estradiol day hCG injection in pregnant patients was 2424.05 pg/ml, ranging from 500 to 10 020 pg/ml. In nonpregnant patients, the mean value was 1967.19 pg/ml, ranging from 124 to 3806 pg/ml. There was a statistically insignificant difference (P value 0.497). Serum estradiol on day of hCG injection reflects the number and health of oocytes, and showed a statistical difference in predicting the response however, it couldn’t prove that value.

The mean value of serum progesterone days 1–3 was 0.554 ng/ml in all patients, 0.587 ng/ml in good responders and 0.318 ng/ml in poor responders, with a statistically insignificant difference (P value 0.244). The mean value of serum progesterone day 1–3 in pregnant patients was 0.485 ng/ml, ranging from 0.1 to 2.1 ng/ml. In nonpregnant patients, the mean value was 0.613 ng/ml, ranging from 0.1 to 1.9 ng/ml. There was a statistically insignificant difference (P value 0.244). The mean value of serum progesterone day hCG was 0.924 ng/ml in all patients, 0.927 ng/ml in good responders and 1.118 ng/ml in poor responders, with a statistically insignificant difference (P value 0.655). The mean value of serum progesterone day hCG injection in pregnant patients was 0.913 ng/ml, ranging from 0.3 to 2.9 ng/ml. In nonpregnant patients, the mean value was 0.939 ng/ml, ranging from 0.3 to 2.5 ng/ml. There was a statistically insignificant difference (P value 0.821).

In a study carried out by Silverberg et al. [22], it was reported that serum progesterone (P4) levels greater than 0.9 ng/ml on the day of hCG administration were associated with decreased PRs in IVF/embryo transfer cycles. Two critical breakpoints were identified: 0.4 and 0.9 ng/ml. Clinical pregnancies occurred in nine of 18 patients in patients with P4 less than 0.4 ng/ml compared with 11 of 81 patients with P4 between 0.4 and 0.9 ng/ml (P = 0.001) and zero of 14 patients with P4 more than or equal to 0.9 ng/ml (P = 0.001). These findings indicate that even modest increases in serum P4 levels (> 0.4 ng/ml) are associated with reduced PRs in IVF/ET cycles. In addition, it appears that the mechanism may not exclusively involve poor oocyte quality [22].

The mean value of serum AMH day 3 was 0.613 ± 0.819 ng/ml in all patients, 0.692 ± 0.819 ng/ml in good responders 0.277 ± 0.505 ng/ml in poor responders, with a statistically significant difference (P value 0.001). In ROC curve analysis, area under the curve was less than 75% (71.71), that is the value of testing AMH in predicting response was low. At a cut-off value of 0.11 ng/ml, the sensitivity was 73.33% and the specificity was 72.85% the likelihood ratio was 2.7. The mean value of basal serum AMH day 3 in pregnant patients was 0.764 ± 0.86 IU/ml, ranging from 0.05 to 4 IU/ml. In nonpregnant patients, the mean value was 0.58 ± 0.904 IU/ml, ranging from 0.05 to 3.8 IU/ml. There was a statistically insignificant difference (P value 0.22). In ROC curve analysis area, under the curve was less than 75% (46.84), that is the value of testing AMH in predicting pregnancy was low. At a cut-off value of 0.3 ng/ml, the sensitivity was 65.38% and specificity was 63.88%. The likelihood ratio was 1.81. Serum AMH day 3 was useful in predicting response; however, it was not significant in predicting pregnancy.

In a study carried out by Gnoth et al. [23] that included 316 patients, a total of 132 oocyte retrievals were performed. A calculated cut-off level less than 1.26 ng/ml AMH alone detected poor respondents (< 4 oocytes) with a sensitivity of 97%, and there was a 98% correct prediction of a normal response in COH if the levels were above this threshold. With levels less than 0.5 ng/ml, a correct prediction of a very poor response (< 2 oocytes) was possible in 88% of cases. Levels of AMH greater than 0.5 ng/ml were not significantly correlated with clinical PRs.

AFC as indicator for ovarian reserve Soliman et al. 131
Their results showed that age and AMH levels are superior parameters predicting ovarian response [23].

To conclude, basal AFC on day (2–5) before down-regulation has a high predictive value in the prediction of OR and it is a cheap and noninvasive test. In addition, age is one of the best markers for ovarian reserve and for us it seems that the age of 38 years is the cut-off point. Moreover, a longer duration of infertility may be indicative of a poor OR.

Acknowledgements

Conflicts of interest
There are no conflicts of interest.

References

Comparison between transfrontal and axial three-dimensional ultrasound acquisition in the visualization of midline structures of the fetal brain
Sherif M.M. Negm and Rasha A. Kamel

Objective
To compare the visualization of the midline structures of the fetal brain as well as visualization of the fastigium of the fourth ventricle and the primary and secondary vermian fissures obtained by three-dimensional (3D) multiplanar reconstruction of volumes acquired from the axial plane with transfrontal 3D acquisition.

Study design
A prospective observational study.

Patients and methods
A total of 127 patients with a normal fetal anomaly scan between 18 and 24 weeks participated in this study. Fetal brain volumes for the multiplanar evaluation were obtained with the transcerebellar plane as the initial plane of acquisition, with the incident ultrasound beam making an angle of about 45° with the cerebral midline. For the transfrontal acquisition, the plane of the midsagittal fetal facial profile was obtained with the ultrasound beam aligned with the frontal suture so as to utilize the metopic suture as an acoustic window.

Results
The acquisition of the fetal brain in the axial plane was successful in 122 cases (96.1%), whereas the transfrontal acquisition was successful in 106 cases (83.4%), with a statistically significant difference between the two methods ($P=0.002$). Visualization of the median plane of the fetal brain by 3D multiplanar reconstruction was adequate in 99 out of the 122 (81.1%) volumes, whereas 94 out of the 106 (88.7%) transfrontal acquisitions resulted in adequate midline images; the difference between the two acquisition methods was not statistically significant ($P=0.12$). There was no statistically significant difference between the two acquisition methods in the visualization of the fastigium of the fourth ventricle or the primary and secondary vermian fissures, which were adequately visualized in 58/122 (47.5%) of the 3D multiplanar reconstructed images and in 62/106 (60.8%) of the transfrontally acquired volumes ($P=0.09$).

Conclusion
Images of the midsagittal plane of the fetal brain obtained by 3D multiplanar reconstruction of volumes acquired from axial plane are easier to acquire than the 3D transfrontal approach and result in comparable image quality, with adequate visualization of the cerebral midline as well as the main landmarks of the cerebellar vermis.

Keywords:
fetal brain, midsagittal plane, multiplanar, three dimensional, three-dimensional ultrasound, transfrontal

Introduction
The standard axial ultrasound views used to evaluate the fetal brain do not adequately display the midline cerebral structures, most notably the corpus callosum and the cerebellar vermis [1], resulting in a low detection rate of congenital anomalies involving these important structures [2,3]. Many authors have thus advocated the use of the transfrontal plane, which is a sagittal section of the fetal head using the metopic suture as an acoustic window, to directly visualize the facial profile and the midline fetal cerebral structure at the same time [4–6]. However, this scanning plane is difficult to obtain at midgestation as most fetuses are in a horizontal line and thus obtaining the proper plane requires considerable ability and, occasionally, transvaginal examination [7].

One of the many advantages of three-dimensional (3D) ultrasound is its ability to manipulate a volume and obtain planes that are different from the one used to acquire it. For this reason, 3D ultrasound has been used in several
studies to obtain reconstructed midline views of the fetal brain from volumes acquired from axial planes [8–10].

The aim of this study is to compare the visualization of the midline structures of the fetal brain, obtained by 3D multiplanar reconstruction of volumes acquired from axial plane with transfrontal 3D acquisition.

Patients and methods
In this prospective observational study, unselected singleton pregnancies between 18 and 24 weeks underwent a fetal anomaly scan by one investigator only (S.M.M.N.) according to the practice guidelines issued by the International Society of Ultrasound in Obstetrics and Gynecology [11]. Patients with medical disorders, multifetal pregnancies as well as pregnancies with obvious fetal structural anomalies were excluded from the study. Healthy patients with an apparently normal anomaly scan were asked to participate in this study. The departmental ethics committee approved the study and an informed consent was obtained from all recruited patients. Ultrasound examinations were carried out with a Voluson 730 Pro (GE Healthcare Austria GmbH & Co., Zipf, Austria) using a transabdominal multifrequency volume probe (RAB 4–8L, GE Healthcare, Austria GmbH & Co., Zipf, Austria). Fetal brain volumes for the multiplanar evaluation were obtained with the fetal head in the axial plane according to the method suggested by Fratelli et al. [12]. Briefly, after optimization of the two-dimensional (2D) image with harmonics set to medium or high, with the transcerebellar plane as the initial axial plane of acquisition, the volume was acquired with the incident ultrasound beam making an angle of about 45° with the cerebral midline so as to avoid acoustic shadowing of the skull base on the posterior fossa. The 3D volume box was adjusted to include the entire fetal head, the acquisition quality was set to maximum, the sweep angle was set to 60–70°, and the upper reference line was set close to the anterior parietal bone. With this method, boxes A, B, and C of the acquired multiplanar images always corresponded to the axial, coronal, and sagittal planes, respectively.

For the transfrontal acquisition, we used the method described by Viñals et al. [13]. The plane of the midsagittal fetal facial profile was obtained with the ultrasound beam aligned with the frontal suture so as to utilize the metopic suture as an acoustic window. Gentle manipulation of the fetal head by the examiner was carried out when required to bring the fetal head into a favorable position. Similar to
the previously described method, the 2D ultrasound image was optimized so as to visualize the corpus callosum and the cerebellar vermis with harmonics set to medium or high. The 3D render box was adjusted to include the entire fetal head, the acquisition quality was set to maximum, and the sweep angle was set at 30°.

For each fetus, at most two acquisitions per 3D technique were obtained and the volumes with the least acoustic shadowing were stored. The stored images were later analyzed offline by either of the two authors (S.M.M.N. or R.A.K.) on a computer using dedicated software (4D view; GE Healthcare). Both authors have several years of experience in the field of fetomaternal ultrasound and are Consultants at the Fetal Medicine Unit at Cairo University. The volumes were manipulated so as to display the clearest image of the sagittal midline plane of the fetal brain, mainly the corpus callosum, the cisterna magna, the third ventricle, the cerebellar vermis, and the cisterna magna. We defined the visualization of the midsagittal plane as adequate when all the anatomical landmarks were identified clearly according to the guidelines set by the International Society of Ultrasound in Obstetrics and Gynecology for carrying out the fetal neurosonogram [14]. The ability to visualize the main landmarks of the cerebellar vermis, mainly the fastigium of fourth ventricle and the primary and secondary vermian cerebellar fissures, was also compared between images obtained by the two methods.

**Statistical analysis**

Normally distributed parameters are presented as mean ± SD and non-normally distributed values are presented as median (range). Normality of data were tested for all continuous variables using the Shapiro–Wilk test. Categorical variables were compared using the χ² or Fisher’s exact test according to cell size. Nonparametric (continuous) variables were compared using the Mann–Whitney U-test. A P value less than 0.05 was considered as statistically significant. Statistical analysis was carried out using Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) and Arcus Quickstat Biomedical version 1.0 (Research Solutions, Cambridge, UK).

**Results**

A total of 127 patients were examined in this prospective observational study. The patients had a mean age of 30.2 years (SD, 3.8; 95% CI, 29.5–30.9), a mean BMI of 24.6 (SD, 1.9; 95% CI, 24.2–24.9), and a mean gestational age of 21.3 weeks (SD, 1.4; 95% CI, 21.0–21.5). Ninety-seven fetuses (76.4%) were in cephalic presentation, whereas the remaining 30 (23.6%) were in breech presentation. The acquisition of the fetal brain in the axial plane was successful in 122 cases (96.1%), whereas the transfrontal acquisition was successful in 106 cases (83.4%), with a statistically significant difference between the two methods (P = 0.002). Table 1 shows that none of the patient or the fetal characteristics had a statistically significant effect on the successful acquisition in either method.

Both the 3D reconstructed images from the axially acquired images as well as the transfrontal 3D images provided detailed visualization of the midline structures of the fetal brain (Figs 1 and 2) in the majority of cases. Visualization of the median plane of the fetal brain by 3D multiplanar reconstruction was considered adequate in 99 out of the 122 (81.1%) volumes acquired from the axial plane, whereas 94 out of the 106 (88.7%) transfrontal acquisitions resulted in adequate midline images; the difference between the two acquisition methods was not statistically significant (P = 0.12). There was no statistically significant difference between the two acquisition methods in the visualization of the fastigium of the fourth ventricle as well as the primary and secondary vermian fissures, which were adequately visualized in 89/122 (47.5%) of the 3D multiplanar reconstructed images from the axial volumes and in 67/110 (60.8%) of the transfrontally acquired volumes (P = 0.09). Images were considered adequate if the corpus callosum could be seen clearly as an anechoic curved structure with an echogenic defining contour overlying and clearly differentiated from the sonoluent cavum septum pellucidum. The contents of the posterior fossa, mainly the cerebellar vermis as an echogenic well-defined structure separated from the occipital bone by the sonoluent cisterna magna, should also be seen. In the cases with inadequate visualization of the midline cerebral structures, shadowing by the cranial bones was the reason for the poor image quality, with poor demarcation of the corpus callosum from the underlying cavum septum pellucidum resulting in a single comma-shaped anechoic structure or an ill-defined posterior fossa.

**Table 1 Effect of the demographic variables on successful axial and transfrontal acquisition**

<table>
<thead>
<tr>
<th></th>
<th>Axial acquisition</th>
<th>Transfrontal acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Successful</td>
<td>Failed</td>
</tr>
<tr>
<td>Patient age [mean (SD)]</td>
<td>30.1 (3.7)</td>
<td>30.0 (3.6)</td>
</tr>
<tr>
<td>BMI [mean (SD)]</td>
<td>24.2 (1.9)</td>
<td>24.8 (2.0)</td>
</tr>
<tr>
<td>Gestational age [mean (SD)]</td>
<td>21.1 (1.4)</td>
<td>21.4 (1.6)</td>
</tr>
<tr>
<td>Cephalic presentation [N (%)]</td>
<td>94 (95.9%)</td>
<td>3 (3.1%)</td>
</tr>
<tr>
<td>Breech presentation [N (%)]</td>
<td>28 (93.3%)</td>
<td>2 (6.7%)</td>
</tr>
</tbody>
</table>

**Discussion**

One of the many advantages of 3D ultrasound is the ability to store the acquired volumes for later offline analysis or even send them to an expert in a different site for analysis by telemedicine. With the aid of specialized software such as 4D view, which we used in the current study, the operator can navigate through a multiplanar display of the volume to construct virtual 2D planes, which are difficult to
acquire using conventional 2D ultrasound such as the midsagittal plane of the fetal brain. The use of postprocessing software such as speckle reduction filters (SRI) can help reduce the tissue speckle pattern and enhance the contrast resolution of the image.

In the present study, we were able to obtain a satisfactory axial view of the fetal head in order to acquire a 3D multiplanar volume in the majority of cases (96.1%), whereas the direct midsagittal view required to acquire a 3D transfrontal volume was obtained in only 83.4% of cases ($P = 0.002$), mainly because of the unfavorable position of the fetal head that could not be manipulated to carry out a proper acquisition, whereas the axial plane, similar to the transthalamic plane used to measure fetal head diameters, was more readily imaged.

Although images with greater detail could have been obtained by transvaginal 3D acquisition as shown by Malinger and Zakut [1], our goal was to try and incorporate fetal neurosonography into the routine transabdominal midtrimester fetal anomaly scan. Furthermore, the transvaginal route would not have been possible in patients with a breech fetus.

Fratelli et al. [12] studied the fetal brain using 3D ultrasound using volumes acquired in the axial view with an angle of 45° between the incident ultrasound beam and the cerebral midline to avoid acoustic shadowing by the skull base on the posterior fossa, which was the method that we adopted in the present study. Their results showed adequate visualization of the midsagittal plane of the fetal brain in 83–87% of examined volumes. They also found good interobserver agreement, with a $\kappa$ value of 0.70. These results are similar to those found in the present study, in which 81.1% of successfully acquired axial volumes resulted in adequately visualized images of the midsagittal plane of the fetal brain. Similar results were obtained by Correa et al. [9], who studied the role of 3D ultrasound in the assessment of the fetal brain and found that acceptable cerebral multiplanar images were obtained in 92% of the cases and that the median sagittal plane could be visualized adequately in 85% of analyzed volumes.

Visentin et al. [15] used conventional 2D ultrasound by the transfrontal view of the fetal head in midtrimester fetuses to visualize the fetal brain in the midsagittal plane. They were able to successfully obtain a transfrontal view in 89% of cases, with good visualization of the midline structures of the fetal brain.

Viñals et al. [13] compared visualization of the midline cerebral structures obtained by 3D multiplanar imaging using an axial acquisition plane with transfrontal 3D acquisition. Measurement of the corpus callosum and

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Figure 2

A three-dimensional transfrontal image of the fetal brain. The midsagittal view of the fetal brain is shown in the A plane image (upper left image), which is the same as the original acquisition plane. CM, cisterna magna; CSP, cavum septum pellucidum; F, fastigium of the fourth ventricle; PF, primary cerebellar fissure; SF, secondary cerebellar fissure; V, cerebellar vermis; 3V, third ventricle.
cerebellar vermis and visualization of the fourth ventricle and the main vermian fissures were also compared. In their study, the midsagittal plane could be obtained in 88 and 87% of the 3D multiplanar and transfrontal images, respectively. They found a good correlation between the two methods in the measurement of the corpus callosum and cerebellar vermis diameters. However, the images obtained by transfrontal acquisition were clearer and more sharply defined than those obtained by the 3D multiplanar method. Also, the primary and secondary fissures of the cerebellar vermis could be detected in 26 and 13%, respectively, of multiplanar images compared to 79 and 52%, respectively, of transfrontal images (P < 0.0001). They attributed the poorer quality of the 3D multiplanar images mainly to acoustic shadowing by the petrous part of the temporal bone, which frequently obscured the fetal brain stem, fourth ventricle, and cerebellar vermis.

The results of Viñals et al. [13] are different from our findings, where we found no statistically significant difference between the two methods in the image quality of the midsagittal plane of the fetal brain (P = 0.12) or in the visualization of the primary and secondary vermian fissures (P = 0.09). Our better results in terms of the midsagittal images obtained by 3D multiplanar acquisition could be attributed to our use of the acquisition method proposed by Fratelli et al. [12], which involves using the transcerebellar plane instead of the transethmoidal plane as the initial plane of acquisition and with the incident ultrasound beam making an angle of 45° with the cerebral midline, thus minimizing the acoustic shadowing of the skull base on the structures of the posterior fossa.

**Conclusion**

Images of the midsagittal plane of the fetal brain obtained by 3D multiplanar reconstruction of volumes acquired from the axial plane are easier to acquire than the 3D transfrontal approach and result in comparable image quality, with adequate visualization of the cerebral midline as well as the main landmarks of the cerebellar vermis.

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**References**

Glycosylated hemoglobin level at 34 weeks in insulin-controlled diabetic pregnancies, relation to fetal outcome
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Objective
To determine the role of glycosylated hemoglobin at 34 weeks’ gestation and onwards in the prediction of fetal outcome in insulin-controlled diabetic pregnancies.

Design
Cohort study.

Setting
Kasr Al-Aini Hospital.

Participants and methods
A total of 50 singleton pregnant women on insulin treatment with gestational age between 34 and 36 weeks were included. All women underwent ultrasound fetometry, and glycosylated hemoglobin level at 34 weeks, amniotic fluid index, placental maturation, and biophysical profile were determined. Doppler ultrasound was also carried out. Follow-up was carried out for all women until delivery and neonates were examined for body weight, APGAR scoring at 1 and 5 min, and blood glucose level (adverse if <45 mg/dl).

Results
There was a statistically significant correlation between HbA1c and BMI, amniotic fluid index, and neonatal outcomes. HbA1c of 7 or higher was found to be a cutoff value for the prediction of prematurity, with area under curve of 91.7% (Fig. 2).

Conclusion
HbA1c may be a useful marker for prematurity in pregnant diabetic women and may correlate with fetal outcome. For the antenatal care of diabetic mothers, it is recommended is to maintain HbA1c less than 7% decrease fetal adverse outcome.

Keywords:
APGAR, glycosylated hemoglobin, maturity, pregnancy

Introduction
Gestational diabetes (GDM) represents a significant health risk for the mother and offspring during pregnancy, delivery, and throughout life. Offspring of women with GDM are at an increased risk for neonatal complications, in particular, macrosomia, large for gestational age, respiratory distress, prematurity, hypoglycemia, polyhydramnios, and death [1]. These offspring are also at a high risk for obesity, insulin resistance, and type 2 diabetes (T2DM) [2]. Thus, the diagnosis and appropriate management of GDM has the potential to markedly reduce neonatal and maternal morbidity and the burden of T2DM.

Glycosylated hemoglobin, as measured by hemoglobin A1C (HbA1c), is used as a marker of glycemic control in patients with type 1 diabetes (T1DM) and T2DM [2]. Notably, the American Diabetic Association has recently recommended that AIC higher than 6.5% may be used as a diagnostic measure for T2DM and that women with AIC higher than 6.5% at their first prenatal visit be diagnosed with T2DM rather than GDM [3]. In the nonpregnant diabetic population, sustained high AIC is associated with increased complications of diabetes [4]. Therefore, optimal AIC levels in pregnancy should be defined by the level of increased risk for adverse pregnancy outcomes. Elevated AIC may be an attractive option for the identification of pregnant women at a high risk for GDM, postpartum T2DM, and macrosomia or delivering an large for gestational age infant. The aim of the present study is to determine a role of glycosylated hemoglobin at 34 weeks’ gestation and onwards in the prediction of fetal outcome in insulin-controlled diabetic pregnancies.

Patients and methods
This prospective cohort study included 50 pregnant women recruited from the Obstetric Outpatient Clinic and Department of Obstetrics & Gynecology at Kasr Al-Aini Hospital. We included singleton pregnant women on insulin treatment with gestational age at the time of inclusion between 34–36 weeks. We excluded women on diet control or those with medical disorders not related to diabetes such as Systemic Lupus Erythematos, thyroid disorders, hypertension, and preeclampsia.
All women underwent ultrasound fetometry, and glycosylated hemoglobin level at 34 weeks, amniotic fluid index, placental maturation, and biophysical profile were determined. Doppler ultrasound was also carried out. Follow-up was carried out for all women until delivery and neonates were examined for body weight, APGAR scoring at 1 and 5 min, adverse neonatal outcome (when the APGAR score is < 7 at 5 min), death either intrauterine or early after birth, neonatal weight more than 4.5 kg, presence of signs of respiratory distress syndrome, and blood glucose less than 45 mg/dl.

Doppler study
Uterine, umbilical, and middle cerebral artery Doppler was carried out by measurement of Doppler indices. The pulsatility index (PI) was used as the test Doppler parameter because PI describes the shape of the velocity wave form much better. (a) Umbilical artery Doppler: All patients were placed in a semirecumbent position with a left lateral tilt, and then the uterine content was scanned to select an area of amniotic cavity with several loops of cord. When the screen showed at least three successive waveforms of similar height, the image was frozen and the Doppler umbilical artery pulsatility index was estimated. (b) Uterine artery Doppler: Doppler examination of the uterine arteries was performed at the same time. Color Doppler was used to identify the apparent crossing of the uterine and iliac vessels. Uterine artery velocimetry was recorded just cranial of the vessel ‘crossing’. Three even subsequent blood flow velocity waveforms were used to calculate PI and to analyze the presence or absence of an early diastolic notch.

Glycosylated hemoglobin
Whole blood was collected by a standard procedure (1 cm). Heparin or edeta was used as an anticoagulant. HbA1c was stable for 7 days at 2 – 8°C. After preparing the hemosytate, where the labile fraction is eliminated, hemoglobins are retained by cation exchange resin, HbA1c is specifically eluted after washing the hemoglobin Aa + b fraction, and quantified by direct photometric reading at 415 nm. Reference values were as follows:

1. HbA1c < 6.3%: very good glycemic control.
2. HbA1c between 6.3 and 7.1%: good glycemic control.
3. HbA1c between 7.1 and 9%: poor glycemic control.
4. HbA1c > 9%: poor glycemic control.

Statistical analysis
The data were coded, entered, and processed on a computer using SPSS (version 16, IBM, New York, USA). Qualitative data are presented as number and percentages whereas quantitative data are presented as mean and SD. Student’s *t*-test was used to assess the statistical significance of the difference between two population means in a study involving independent samples. A P-value was considered significant if less than 0.05.

The Pearson correlation coefficient was used to determine the relation between the quantitative parameters. Logistic regression analysis was used to assess the effect of quantitative parameter on an outcome. ROC curve was used to assess the sensitivity and specificity of factor in predicting the outcome.

Glycosylated Hb and fetal outcome

Results
The present study included women ranging in age from 19 to 35 years, mean ± SD (26.94 ± 4.497); the patient’s BMI ranged from 21 to 33, mean ± SD (25.04 ± 3.110). Table 1 shows other characteristics of our participants. No statistically significant relation was found between HbA1c and age of the studied patients, whereas there was a statistically significant correlation between HbA1c and BMI, amniotic fluid index, and neonatal outcomes (Table 2). HbA1c of 7 or higher was found to be a cutoff value for prediction of prematurity with an area under curve of 91.7% (Fig. 1); also, this cutoff value was used for the prediction of polyhydramnios, macrosomia, neonatal hypoglycemia (Fig. 2), respiratory distress syndrome, and cesarean section rate (with area under the curve of 77.5, 72.6, 98.3, 98.3, and 87.1, respectively).

Discussion
In the USA, the prevalence of GDM may range from 1 to 14% of pregnancies [3]. GDM generally has few symptoms and it is most commonly diagnosed by screening during pregnancy. Diagnostic tests detect inappropriately high levels of glucose in blood samples [5].

Glycosylated hemoglobin, as measured by HbA1c, is used as a marker of glycemic control in patients with T1DM and T2DM.

Table 1 Descriptive statistics for the parameters studied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin dose</td>
<td>30</td>
<td>90</td>
<td>41.80</td>
<td>13.92</td>
</tr>
<tr>
<td>FW (kg)</td>
<td>1.6</td>
<td>4.9</td>
<td>3.09</td>
<td>0.81</td>
</tr>
<tr>
<td>AFI</td>
<td>6</td>
<td>28</td>
<td>17.40</td>
<td>5.47</td>
</tr>
<tr>
<td>BPD</td>
<td>4</td>
<td>8</td>
<td>7.40</td>
<td>1.01</td>
</tr>
<tr>
<td>UTA PI</td>
<td>0.6</td>
<td>1.2</td>
<td>0.78</td>
<td>0.14</td>
</tr>
<tr>
<td>MCA PI</td>
<td>1.4</td>
<td>1.95</td>
<td>1.81</td>
<td>0.17</td>
</tr>
<tr>
<td>UMA PI</td>
<td>0.81</td>
<td>1.25</td>
<td>0.92</td>
<td>0.13</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.8</td>
<td>13</td>
<td>8.17</td>
<td>1.98</td>
</tr>
<tr>
<td>FBS</td>
<td>70</td>
<td>135</td>
<td>96.46</td>
<td>19.76</td>
</tr>
<tr>
<td>2hPPS</td>
<td>120</td>
<td>280</td>
<td>186.08</td>
<td>59.72</td>
</tr>
<tr>
<td>APGAR1</td>
<td>4</td>
<td>8</td>
<td>6.65</td>
<td>1.41</td>
</tr>
<tr>
<td>APGARS</td>
<td>5</td>
<td>10</td>
<td>7.87</td>
<td>1.84</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2</td>
<td>5.2</td>
<td>3.30</td>
<td>0.74</td>
</tr>
<tr>
<td>Glucose</td>
<td>25</td>
<td>85</td>
<td>49.06</td>
<td>20.36</td>
</tr>
<tr>
<td>Delivery age (weeks)</td>
<td>35</td>
<td>38</td>
<td>36.69</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Table 2 Correlation between HbA1c with age, BMI, AFI, BMI fetal weight, birth weight, neonatal glucose, and APGARS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>R</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.244</td>
<td>0.088</td>
</tr>
<tr>
<td>BMI</td>
<td>0.432**</td>
<td>0.002</td>
</tr>
<tr>
<td>AFI</td>
<td>0.301*</td>
<td>0.034</td>
</tr>
<tr>
<td>FW (kg)</td>
<td>0.426**</td>
<td>0.003</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>0.308*</td>
<td>0.033</td>
</tr>
<tr>
<td>Neonatal glucose</td>
<td>-0.552**</td>
<td>0.000</td>
</tr>
<tr>
<td>APGARS</td>
<td>-0.689**</td>
<td>0.000</td>
</tr>
</tbody>
</table>

AFI, amniotic fluid index; FW, fetal weight.
*Correlation is significant at the 0.05 level (two-tailed).
**Correlation is significant at the 0.01 level (two-tailed).
In the present study, we aimed to evaluate its use as a marker in gestational diabetic women at 34 weeks and follow them up. Interestingly, the present study observed a positive relation between glycosylated hemoglobin and adverse fetal outcome; that is, when HbA1c more than 7% at 34 weeks, there was increase in the frequency of adverse fetal outcomes including respiratory distress syndrome, macrosomia, polyhydramnios, prematurity, and neonatal hypoglycemia.[1]

The results of the current study showed that the sensitivity of HbA1c more than 7% for predicting polyhydramnios was 100% and the specificity was 57.14%. The incidence of large for gestational age was 38% and this was in agreement with the result of Yang et al. [6], who reported a 40% in Pregnancy Outcomes of Births in Nova Scotia from 1988 to 2002 in Women with and Without Pre-gestational Diabetes.

The study also found a highly statistically significant positive relation between HbA1c with fetal weight and a significant positive relation with birth weight ($P = 0.003$ and $0.033$, respectively), which was in agreement with Katon et al [7], who reported that the association of A1C and birth weight was stronger when antepartum A1C was measured after a diagnosis of GDM (at 34 weeks).

The results of the current study showed that the sensitivity of HbA1c more than 7% for predicting macrosomia was 82% and the specificity was 63%. In this study, the incidence of hypoglycemia was 40% and there was a highly statistically significant relation ($P < 0.005$) between HbA1c and neonatal glucose level, yielding an area under the curve of 98.3. This was in agreement with Arumugam and Abdul Majeed [8], who reported that HbA1c levels in late pregnancy are good predictors of hypoglycemia in the newborn, yielding an area under the curve of 0.99. In simple terms, what this means is that if there were two babies who were randomly selected, one with hypoglycemia and one without, the probability that the hypoglycemic neonate would have shown an abnormally high maternal HbA1c would be around 99% [9]. In this study, there was no statistically significant relation between HbA1c and Doppler and this in agreement with Pietryga et al. [10], who concluded that there was no correlation between long-term maternal glycemic control (HbA1c) and changes in blood flow velocity in placental circulation in pregnancy complicated by diabetes mellitus.

Conclusion
HbA1c may be a useful marker for prematurity in pregnant diabetic women. For the antenatal care of diabetic mothers, we recommend maintaining HbA1c less than 7% to decrease fetal adverse outcome. For further confirmation of the above results, we need to study a larger number of patients.

Acknowledgements
Conflicts of interest
There are no conflicts of interest.

References
Evaluation of the atherogenic role of lipoproteins and oxidized low-density lipoprotein in pre-eclampsia

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Objectives
The aim of this study was to evaluate the levels of lipid parameters and oxidized low-density lipoprotein (ox-LDL) and compare them in normal and pre-eclamptic pregnant women as well as to determine the relative risk of developing pregnancy-induced hypertension disorders in patients in whom ox-LDL levels are elevated.

Participants and methods
Singleton pregnant women (18–38 years old) were recruited and divided into two groups: 26 women with pre-eclampsia and 21 women with normal uncomplicated pregnancies. Blood and urine samples were taken from all the participants; different lipid parameters including triglycerides, cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were determined. In addition, urine protein and ox-LDL were assessed.

Results
The two groups studied were matched in terms of maternal age, BMI, and gestational age. Pre-eclamptic patients had significantly higher blood pressure compared with the women with normal pregnancies (control). Of the measured lipid parameters, significantly higher values of triglycerides, very low-density lipoprotein-C, and ox-LDL were found in the women in the pre-eclampsia group compared with the control group (\(P=0.003\) and 0.049, respectively). None of the measured lipid parameters showed a significant association when correlated to ox-LDL. The calculated cut-off for ox-LDL in our study was 36 ng/ml. Patients with high ox-LDL levels during pregnancy had 1.7 times higher risk for developing pre-eclampsia than women with normal pregnancies.

Conclusion
Dyslipidemia and oxidative stress may play an important role in the pathogenesis of pregnancy-induced hypertensive disorders. Thus, our findings may be relevant for understanding the pathophysiology of pre-eclampsia and showed that there is a need for further research work to establish the role of lipid-modifying regimens, lifestyle modifications, and antioxidant therapy to reduce the risk of developing such disorders.

Keywords:
dyslipidemia, lipids, oxidized low-density lipoprotein, pre-eclampsia, pregnancy

Introduction
Pre-eclampsia is a common multisystem pregnancy disorder in which the diagnosis is made on the basis of the presence of hypertension and proteinuria, affecting 3–5% of all pregnant women. Pre-eclampsia occurs \textit{de novo} in late pregnancy after the 20th week of gestation, and severe pre-eclampsia may be a serious threat to the mother and the fetus. This disorder is a major cause of prenatal and maternal morbidity and mortality worldwide [1].

The etiology of pre-eclampsia is still unknown and multiple factors have been implicated in its pathogenesis. Metabolic syndrome, especially insulin resistance, and pre-eclampsia have been well examined in previous research [2,3]. Insulin resistance is common in pregnancy, as are aberrant lipid metabolism, higher fasting glucose level, and increased insulin secretion. These metabolic changes are likely to have evolved to fulfill the metabolic demands of a growing fetus, but these changes are augmented in pre-eclampsia [4].

Previous studies have shown that in pre-eclampsia, plasma lipids increase markedly beyond the levels found in normal pregnancies [5,6]. It has been proposed that such lipid changes may play a role in the endothelial damage characteristic of pre-eclampsia. Given the physiological role of gestational hyperlipidemia in supplying both cholesterol and triglycerides to the rapidly developing fetus, it is conceivable that pregnancies complicated by intrauterine growth retardation show abnormal lipid protein metabolism in an attempt to compensate for the placental insufficiency. This mechanism has been proposed to be responsible for the higher triglyceride concentrations observed in women with pre-eclampsia [7].

Diffuse vascular endothelial dysfunction, secondary to oxidative stress, is an important pathological characteristic...
of pre-eclampsia. Oxidative conversion of low-density lipoproteins (LDLs) into oxidized-LDL (ox-LDL) is considered an important step in the transformation of macrophages into lipid-laden foam cells destined to develop into early atherosclerotic-like lesions [8].

Small dense LDL is also increased in the plasma of women with pre-eclampsia. Small dense LDLs are more susceptible to oxidation, resulting in the generation of ox-LDL, which can bind to the lectin-like oxidized-LDL receptor-1 (LOX-1) on endothelial cells. LOX-1 is a type II membrane protein cell surface receptor found on endothelial cells, vascular smooth muscle cells, and monocyte macrophages. LOX-1 is expressed in atherosclerotic lesions in humans and has also been shown to be elevated in hypertensive rats [9]. LOX-1 is responsible for the binding, uptake, and degradation of ox-LDL. One recent study has reported elevated LOX-1 expression in the placenta of women with pre-eclampsia. However, the expression, regulation, and significance of LOX-1 in the maternal systemic vasculature of pre-eclampsia remain unknown [10].

The aims of the present study are to measure the serum levels of cholesterol, triglyceride, high-density lipoprotein (HDL), LDL, and ox-LDL in pre-eclamptic pregnancy and to compare them with those in normal pregnancy.

**Participants and methods**

The present study was conducted from December 2009 to January 2011 among women with pre-eclampsia according to the 10 current American College of Obstetricians and Gynecologists guidelines (American College of Obstetricians and Gynecologists, 1996), which defined pre-eclampsia as sustained pregnancy-induced hypertension with proteinuria. Pre-eclampsia was defined as persistent (≥6 h) blood pressure of at least 140/90 mmHg occurring after 20 weeks of gestation. Proteinuria was defined as a urine protein concentration of 30 mg/dl or higher (or 1+ on a urine dipstick or higher) in at least two random specimens collected at least 4 h apart.

Two groups of 26 women with pre-eclampsia and 21 controls with uncomplicated pregnancies matched for age, gestational age, weight, and BMI were recruited. We excluded women with pregnancies associated with diabetes mellitus, hepatic, renal, metabolic, cardiovascular diseases, chronic or transient hypertension, thyrotoxicosis, and hemophilia from our study. Also, pregnant women with a previous history of gestational diabetes or those on chronic medications were excluded from the study.

**Data and sample collection**

During participants’ antenatal care visits, we administered structured interview questionnaires to collect information on maternal sociodemographic, medical, reproductive, and lifestyle characteristics. All interviews were conducted in Arabic. BMI and the measure of adiposity were calculated as weight (kg) divided by height (m²). Nonfasting blood samples were collected in dry 10 ml vacutainer tubes during the visit. Specimens were centrifuged at 3000g; serum was separated and frozen at –70°C until analysis.

**Analytical methods**

Serum cholesterol and triglyceride concentrations were measured enzymatically using assays on a Hitachi 917 chemistry analyzer (Roche Diagnostics, Berlin, Germany) with a colorimetric procedure. HDL-C was assayed using a homogenous enzymatic assay on a Hitachi 917 chemistry analyzer (Roche Diagnostics). LDL-cholesterol (LDL-C) was calculated using the Friedewald formula [11]. From the previous assays, the HDL/LDL and cholesterol/HDL ratios were calculated. Plasma ox-LDL concentrations were measured using commercial ELISA assay kits (Immundiagnostik AG, Bensheim, Germany).

**Statistical analysis**

All analyses were performed using SPSS statistical software version 10 (IBM, Chicago, Illinois, USA). All continuous variables were presented as mean ± SD. All reported confidence intervals were calculated at the 95% level. Differences between groups were evaluated using Student's unpaired t-test and, when a variable was not normally distributed, the Mann–Whitney U-test and Wilcoxon H-tests were used. The relationship between the variable was explored using the Pearson correlation test. The association of ox-LDL with the maternal plasma lipid profile was estimated using Spearman’s correlation coefficients. The cutoff for ox-LDL was estimated using the receiver operating characteristic (ROC) curve, area under the curve, and the best sensitivity/specificity was determined to calculate the cutoff. Logistic regression procedures were used to calculate odds ratios and 95% confidence intervals (CIs). To estimate the relative association between pre-eclampsia and levels of maternal ox-LDL, we categorized each study group into Healthy and Cases on the basis of the level of the ox-LDL cut-off value determined by the ROC curve.

**Results**

The demographic and clinical characteristics of pre-eclampsia cases and controls are shown in Table 1. The two groups studied were matched for maternal age (18–38 years old), BMI, and gestational age at sampling. Systolic and diastolic blood pressures were significantly higher in women with pre-eclampsia (mean 163 and 105, respectively) than those in the control group (mean 122 and 77, respectively) (P<0.001). Infants of pre-eclamptic women had significantly lower Apgar scores for their pregnancy outcomes when compared with those of women with normal pregnancies.

Pregnant women with a complication of pre-eclampsia had significantly higher mean triglyceride and very low-density lipoprotein-C (VLDL-C) concentrations, relative to the control group (P = 0.003 and 0.049, respectively). In contrast, there were no significant differences in the mean concentrations of total cholesterol, HDL-C and LDL-C, and risk ratios between the cases and the...
controls ($P>0.05$) as determined using the $t$-test as shown in Table 2. Significantly higher ox-LDL values were found in the pre-eclampsia group compared with the control group using the Mann–Whitney $U$-test ($P = 0.044$).

We next examined the correlations between maternal plasma ox-LDL concentrations and lipid profile for pre-eclampsia cases and controls Table 3. Ox-LDL concentrations were nonsignificantly positively correlated with cholesterol ($r = 0.043$) and triglycerides ($r = 0.072$) ($P > 0.05$) in both pre-eclampsia cases and the controls. Ox-LDL concentrations were nonsignificantly inversely correlated with HDL-C ($r = -0.26$) and LDL-C ($r = -0.023$) ($P > 0.05$). The calculated cut-off value for ox-LDL was 36 ng/ml.

Ox-LDL levels were higher in women with pre-eclampsia than those of the women in the control group. The overall risk odds ratio was 1.750 (CI ranging from 0.364 to 8.424); the ratio was 1.375 for the cases, with CI ranging from 0.547 to 3.459, and 0.786 for the healthy controls, with CI ranging from 0.405 to 1.525 (Table 4 and Fig. 1).

**Discussion**

Experimental models indicated that increased ox-LDL is capable of inducing many endothelial changes of potential relevance to pre-eclampsia [12]. However, it remains unclear whether circulating ox-LDL is increased in pre-eclampsia. Increased autoantibodies to an epitope of ox-LDL, which is considered an indirect marker of ox-LDL, have been found in women with pre-eclampsia relative to those with a normal pregnancy [13], although negative reports also exist [14,15]. The role and status of serum lipids and ox-LDL in pregnant women are still being discussed. The aims of the present study are to measure the serum levels of cholesterol, triglyceride, HDL, LDL, and ox-LDL in women with pre-eclampsia and to compare these with those of women with normal pregnancies.

In the present study, there was no significant difference ($P > 0.05$) in the serum levels of cholesterol, HDL-C, LDL-C, and risk ratios between women with pre-eclamptic pregnancies and those with normal pregnancies. Our results are in agreement with those of some studies [6,16], but not with other research works that have reported significant increases in cholesterol, HDL-C, and LDL-C [17,18]. These findings may be because of differences in races and nutrition status. It should be noted that all blood samples were taken from preeclamptic women at their entry to Obstetrics and Gynecology department to avoid any possible effect of medications on the results of our study.

Significantly higher values ($P < 0.05$) triglycerides and VLDL-C were found in the pre-eclampsia group when compared with normal pregnant women. Some previous studies showed that the most significant damage in the lipid profile in normal pregnancy is serum hypertriglyceridemia, which may be as high as two- to three-fold in the third trimester compared with the levels in nonpregnant women. The principal modulator of this hypertriglyceridemia is estrogen as pregnancy is associated with hyperestrogenemia. Estrogen induces hepatic biosynthesis of endogenous triglycerides, which is carried by VLDL-C. This process may be modulated by hyperinsulinism found in pregnancy. Serum triglyceride and VLDL-C concentrations were also found to increase significantly more in toxemia of pregnancy in our study, which is in agreement with the findings of many workers [17,19]. The above-mentioned associations along with increased endothelial triglyceride accumulation may result in endothelial cell dysfunction in gestosis. Increased triglycerides, found in pregnancy-induced hypertension, is likely to be deposited in predisposed vessels, such as the uterine spiral arteries, and contributes to endothelial dysfunction, both directly and indirectly, through the generation of small, dense LDL. Moreover, this hypertriglyceridemia may be associated with hypercoagulability [20].

The values of ox-LDL in our study were significantly increased ($P < 0.05$) in pre-eclamptic women than in normal pregnancies. Oxidation of LDL is commonly believed to be essentially involved in the pathogenesis of atherosclerosis, which is known to be the primary cause of cardiovascular disease and stroke. Recent studies have reported that ox-LDL impairs trophoblast invasion in vitro, suggesting its role in the regulation of uterine spiral artery remodeling in vivo [21]. Reduced trophoblast invasion and atherosclerotic placental changes, described as ‘acute atherosis’, are hallmarks in the pathogenesis of pre-eclampsia and intrauterine growth retardation. Some efforts have been made to evaluate ox-LDL levels in PE, which have yielded inconsistent data; although in two studies the ox-LDL concentration was found to be significantly increased in comparison with normal healthy pregnant controls [22,23], one research group did not find a significant difference, but reported a 2.9-fold increased risk for PE in women who had elevated ox-LDL levels [24]. Only the group of Rajmakers et al. [25] reported lower ox-LDL concentrations in PE.

None of the lipid and lipoprotein parameters showed a significant association ($P > 0.05$) with ox-LDL, while total cholesterol, triglycerides, and VLDL-C showed positive correlations with ox-LDL, maternal serum LDL-C and HDL-C showed negative correlations. In one research work [24], lipid parameters showed significant

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**Table 1 Demographic and clinical data of the studied groups**

<table>
<thead>
<tr>
<th>Items</th>
<th>Pre-eclampsia group</th>
<th>Control group</th>
<th>P value ($t$-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>34.2 ± 2.3</td>
<td>32.1 ± 2.1</td>
<td>0.78</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational (age/weeks)</td>
<td>272 ± 5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apgar score (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.1 ± 1.2</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.8 ± 1.4</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm/Hg)</td>
<td>163.7 ± 13.8</td>
<td>122 ± 3.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diastolic BP (mm/Hg)</td>
<td>105 ± 10</td>
<td>77 ± 4.3</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

BP, blood pressure.
associations with ox-LDL; this controversy results with our findings may have been because of difference in number of participants. The ROC curve constructed for the estimation of the cut-off for ox-LDL in pre-eclampsia was 36 ng/ml in our study.

In the present study, it was found that women with increased ox-LDL concentrations had a 1.7-fold increased risk of pre-eclampsia, compared with women who had lower ox-LDL levels. Our results are in agreement with those of some research works [24,26] and some results of autoantibodies to ox-LDL (an indirect marker of in-vivo ox-LDL) from cross-sectional case–control studies [13,27]. However, there has been only one published negative report on maternal plasma ox-LDL concentrations in relation to the risk of developing pre-eclampsia [25]. In that study, women with pre-eclampsia had lower ox-LDL concentrations than gestational-age-matched normotensive pregnant controls. The authors attributed this inverse association to the higher levels of autoantibodies to ox-LDL, which might be responsible for the rapid clearance of ox-LDL.

### Table 2 Lipid parameters and oxidized low-density lipoprotein values of the studied groups

<table>
<thead>
<tr>
<th>Items</th>
<th>Pre-eclampsia group</th>
<th>Control group</th>
<th>(P) value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>179.25 ± 55.243</td>
<td>186.64 ± 45.502</td>
<td>0.718</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>207.69 ± 84.467</td>
<td>210.64 ± 69.818</td>
<td>0.003</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>41.53 ± 16.893</td>
<td>21.528 ± 13.924</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>44.31 ± 9.555</td>
<td>46.45 ± 5.484</td>
<td>0.510</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>89.56 ± 51.673</td>
<td>118.64 ± 40.562</td>
<td>0.131</td>
</tr>
<tr>
<td>Cholesterol/HDL ratio</td>
<td>4.04 ± 5.782</td>
<td>4.018 ± 8.297</td>
<td>0.621</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>2.02 ± 5.408</td>
<td>2.554 ± 7.386</td>
<td>0.172</td>
</tr>
<tr>
<td>Ox-LDL (ng/ml)</td>
<td>12.56 ± 198.654</td>
<td>0.00 ± 108.838</td>
<td>0.044</td>
</tr>
</tbody>
</table>

C, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ox-LDL, oxidized-LDL; VLDL, very low-density lipoprotein.

*Median concentrations.

### Table 3 Correlations of oxidized low-density lipoprotein with different lipid parameters in both the groups (Spearman’s correlation)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient (r)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>0.043</td>
<td>0.830</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.072</td>
<td>0.719</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-2.26</td>
<td>0.258</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.023</td>
<td>0.908</td>
</tr>
</tbody>
</table>

C, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

### Table 4 Risk estimation by odds ratios and 95% confidence interval of pre-eclampsia according to oxidized low-density lipoprotein subgroups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Odds ratio</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-eclampsia/Control</td>
<td>1.750</td>
<td>0.364</td>
<td>8.424</td>
</tr>
<tr>
<td>For cohort odds Cases (pre-eclampsia/Control)</td>
<td>1.375</td>
<td>0.547</td>
<td>4.369</td>
</tr>
<tr>
<td>For cohort odds Healthy (pre-eclampsia/Control)</td>
<td>0.786</td>
<td>0.405</td>
<td>1.525</td>
</tr>
</tbody>
</table>

Receiver operating characteristic (ROC) curve for the oxidized low-density lipoprotein. Area under the curve = 0.267, 95% confidence interval = 0.05–0.484, Cutoff (ox-LDL) = 36 ng/ml (40% sensitivity and 50% specificity).

### Conclusion

Compared with normal pregnant women with similar gestational age and BMI, with no significant difference in maternal age, we observed that pre-eclamptic women had higher blood pressure and increased triglycerides, VLDL-C, and ox-LDL during pregnancy. Increased ox-LDL in pregnancy is associated with a higher risk of developing pregnancy-induced hypertensive diseases. Our results confirm the role of dyslipidemia and oxidative stress in the pathogenesis of pregnancy-induced hypertensive disorders. There is a need for further research work to establish the role of diet, lifestyle modifications, and antioxidant therapy to reduce the risk of development of such disorders.
Acknowledgements
Conflicts of interest
There are no conflicts of interest.

References