Impact of oestradiol and inhibin A concentrations on pregnancy rate in in-vitro oocyte maturation

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The purpose of the present study was to analyse the results of maturation of oocytes obtained in unstimulated normal women after a leading follicle of 10 mm diameter and an endometrium of at least 5 mm thickness were observed at ultrasound. The serum concentrations of oestradiol and inhibin A were analysed from day 3 to the day of aspiration and retrospectively evaluated. A total of 75 normal regular cycling women referred for IVF/intracytoplasmic sperm injection (ICSI) because of male factor and/or tubal disease was included (n = 87 consecutive cycles). The oocytes were aspirated transvaginally and matured for 28–36 h. ICSI was performed on all metaphase II oocytes and they were cultured to day 2 or 3 after insemination, at which time suitable embryos (maximum two) were replaced into the women. Eleven singleton pregnancies with a live fetus were obtained after transfer in 63 cycles (pregnancy rate of 13% per aspiration and 17% per transfer). Nine healthy children have been born and the remaining two pregnancies miscarried in the eighth to ninth gestational week. The serum concentrations of oestradiol on day 3 and on the day of aspiration were available in 85 cycles, and in 57 of these an increase of 100% was detected on the day of aspiration. Significantly more pregnancies were observed in these cycles compared with cycles without an increase in the concentration of oestradiol (19 versus 0% per aspiration, 24 versus 0% per transfer, P < 0.02). Further improvement in pregnancy rate was obtained if aspiration was performed after increase in inhibin A concentration (24 versus 0% per aspiration and 33 versus 0% per transfer, P < 0.02) (n = 83 cycles, where inhibin concentrations were available, and n = 42 cycles, where an increase of 80% was detected on the day of aspiration compared with day 3). Timing of aspiration may improve the developmental potential of immature oocytes.

Key words: human oocytes/maturation in vitro/pregnancy rate/timing of aspiration

Introduction

Immature oocyte retrieval combined with in-vitro maturation (IVM) is an attractive alternative to conventional in-vitro fertilization (IVF). Although it is possible to mature and fertilize human oocytes obtained from unstimulated cycles, the success rate in terms of pregnancy rate after IVM has in general been low (Cha et al., 1991; Trounson et al., 1994; Barnes et al., 1996; Russell et al., 1997; Mikkelsen et al., 1999). There are several possible explanations for the poor developmental capacity of these oocytes, including suboptimal culture conditions during IVM or that the oocytes themselves were defective due to inadequate cytoplasmic maturation (Moor et al., 1998).

While some studies have focused on improved culture media (Hwu et al., 1998; Trounson et al., 1998) and the time interval for maturation before insemination (Smith et al., 2000), others have tried to optimize the quality of the oocyte. The administration of oestradiol from day 3 to the day of aspiration gave a lower maturation rate and impaired embryonic development compared to late priming with oestradiol, possibly owing to feedback inhibition of dominant follicle selection (Russell et al., 1997). A possible positive effect of FSH priming has been evaluated, but results are conflicting (Wynn et al., 1998; Trounson et al., 1998; Mikkelsen et al., 1999).

The importance of the timing of oocyte retrieval in human IVM is presently unclear.

The maturation potential of the oocytes may be affected by dynamic physiological changes which occur within the follicle, and these factors may change from the early to the late follicular phase, before and after selection of a dominant follicle respectively (Fauser and van Heusden, 1997).

It has been considered that the dominant follicle can be recognized by ultrasound when the diameter has reached 10 mm (Pache et al., 1990; Fauser and van Heusden, 1997). In a pilot study we observed an improved pregnancy rate when oocytes were obtained after a follicle of 10 mm was demonstrated (Mikkelsen et al., 1999). Others, however, have found that once selection of the leading follicle has occurred, the number of retrieved oocytes and their ability to develop into blastocysts is impaired (Cobo et al., 1999). Some investigators have found a detrimental effect of early atresia on the oocytes (Russell, 1998), while others were not able to demonstrate this (Thornton et al., 1999).

Growth and development of the dominant follicle is correlated with secretion of both oestradiol and inhibin A into the circulation (Lockwood et al., 1998). The follicular concentration of inhibin A increases while that of inhibin B decreases with increasing follicular size (Magoffin and Jakimik, 1997; Lau et al., 1999). This is consistent with the increasing and decreasing concentrations of inhibin A and B respectively in serum seen as the pre-ovulatory dominant follicle matures (Groome et al., 1996). The increase in serum concentrations of inhibin A has been found to parallel that of...
oestradiol but with a delay of 1–2 days (Groome et al., 1996; Magoffin and Jakimiuk, 1997; Lahlau et al., 1999).

The purpose of the present study was to analyse the results of maturation of immature oocytes obtained in unstimulated normal women after a leading follicle of 10 mm diameter and an endometrium of at least 5 mm thickness were demonstrated at ultrasound. The concentrations of oestradiol and inhibin A in serum were analysed retrospectively from day 3 to the day of aspiration to investigate their potential as possible biochemical markers for oocyte quality. The quality of the oocytes was evaluated by maturation, fertilization, cleavage and pregnancy rates.

Materials and methods

A total of 75 women with normal regular cycles referred for IVF/ intracytoplasmic sperm injection (ICSI) due to male factor and/or tubal disease was included (n = 87 cycles). The women were at least 18 and at most 37 years of age and had normal ovulatory cycles with a mean duration 26–35 days and a body mass index (BMI) 18–29 kg/m². Excluded were all patients with infertility caused by endocrine abnormalities such as hyperprolactinaemia and patients with expected low ovarian reserve [evaluated on day 3 by an antral follicle count of <3 follicles at 2–5 mm diameter and/or an increased concentration of follicle stimulating hormone (FSH) >15 IU (Scott et al., 1989) and/or a decreased concentration of inhibin B <45 pg/ml (Seifer et al., 1997)]. Also excluded were patients who had previously failed to conceive with conventional IVF after ≥3 attempts, and patients with possible poor quality of the oocytes, i.e. patients with a low <20% cleavage rate at conventional IVF, and women with polycystic ovarian syndrome (ultrasound examination showed >10 follicles in one plane and hormone analysis showed elevated LH/FSH ratio or elevated androgens).

A transvaginal ultrasound was performed on cycle day 3. In the case of an ovarian cyst the cycle was cancelled. The second ultrasound examination was performed on day 6–7, and the following days ultrasound was performed daily or with an interval of 2–3 days depending on the size of the follicles. Ovum retrieval was scheduled when a leading follicle reached 10 mm in diameter and the endometrium showed pattern I or II (Grunfeld et al., 1991) with a thickness of at least 5 mm. Oocytes were recovered from visible follicles of >3 mm diameter. The number of follicles aspirated corresponded to the number of follicles on day 3, but the recovery rate of oocytes from unstimulated ovaries was reduced compared to conventional IVF, where aspiration is performed on follicles at least 18–20 mm in diameter after ovarian stimulation. The number of oocytes obtained in the present study is less than previously reported (Russell et al., 1998). Serum concentrations of oestradiol were obtained on each day ultrasound was performed and on the day of aspiration. Serum oestradiol and inhibin concentrations on day 3 were compared to those on the day of aspiration. Oocytes obtained from cycles with a detected increase of 100% in oestradiol and of 80% in inhibin A were compared to oocytes without these magnitudes of increase in the serum concentrations of these hormones. The hormone values are evaluated retrospectively. By analysing the final pregnancy rate in relation to the concentration of oestradiol and inhibin A we found acceptable pregnancy rates, when an increase of 100% of the concentration of oestradiol and 80% of the concentration of inhibin A was chosen. We might have chosen a 100% increase for both oestradiol and inhibin A, as this would have increased the pregnancy rate even further in the group with a detected increase in inhibin A but we decided to err on the side of caution.

Oocyte recovery, maturation, fertilization and embryo culture

Oocyte recovery was performed transvaginally with a single lumen needle connected to a syringe to induce the aspiration vacuum. The follicular aspirates were transferred into tubes containing Ham F-10 medium with heparin at 37°C (Life Technologies, Copenhagen, Denmark). Follicular aspirates were filtered (Falcon 1060; 70 mm mesh size) to remove erythrocytes and small cellular debris. Oocytes with signs of atresia (dark or shrunk, irregular cytoplasm) or mechanical damage were discharged. Healthy-appearing oocytes were matured in TCM 199 medium (Sigma, Roedovre, Denmark) or in a specific IVM medium (Medi-Cult, Copenhagen, Denmark). The culture medium was supplemented with sodium pyruvate 0.3 mmol/l, 1500 IU/ml penicillin G, 50 mg/ml streptomycin sulphate, oestradiol 1 mg/ml (all from Sigma), recombinant FSH 0.075 IU/ml.

### Table I. The maturation, fertilization and cleavage rates of oocytes obtained after a detected increase in oestradiol (n = 85 cycles)

<table>
<thead>
<tr>
<th>Oestradiol</th>
<th>No. aspirations</th>
<th>No. transfers</th>
<th>No. oocytes</th>
<th>For IVM (%)</th>
<th>MII (%)</th>
<th>2PN (%)</th>
<th>Cleavage (%)</th>
<th>No. pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;100% increase</td>
<td>57</td>
<td>45</td>
<td>342</td>
<td>258</td>
<td>160 (62)</td>
<td>124 (78)</td>
<td>108 (87)</td>
<td>11b (19)</td>
</tr>
<tr>
<td>&lt;100% increase</td>
<td>28</td>
<td>17</td>
<td>161</td>
<td>115</td>
<td>68 (59)</td>
<td>48 (71)</td>
<td>43 (90)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*aOnly cycles in which oestradiol concentration was available on both day 3 and on the day of aspiration were included.

bP < 0.02, Fisher’s exact test.

IVM = in-vitro maturation; 2PN = two pronuclei; MII = metaphase II.

### Table II. The maturation, fertilization and cleavage rates of oocytes obtained after a detected increase in both oestradiol and inhibin A (n = 83 cycles)

<table>
<thead>
<tr>
<th>Inhibin A</th>
<th>No. aspirations</th>
<th>No. transfers</th>
<th>No. oocytes</th>
<th>For IVM (%)</th>
<th>MII (%)</th>
<th>2PN (%)</th>
<th>Cleavage (%)</th>
<th>No. pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;80% increaseb</td>
<td>42</td>
<td>30</td>
<td>262</td>
<td>191 (73)</td>
<td>116 (60)</td>
<td>94 (81)</td>
<td>81 (86)</td>
<td>10b</td>
</tr>
<tr>
<td>&lt;80% increaseb</td>
<td>41</td>
<td>30</td>
<td>246</td>
<td>182 (74)</td>
<td>112 (62)</td>
<td>78 (69)</td>
<td>70 (89)</td>
<td>0</td>
</tr>
</tbody>
</table>

*aAlso showed oestradiol increase of at least 100%.

bP < 0.02 Fisher’s exact test.

IVM = in-vitro maturation; 2PN = two pronuclei; MII = metaphase II.
Table III. Clinical variables in the cycles with a detected increase in oestradiol and inhibin A on the day of aspiration compared to cycles without a corresponding increase (\(n = 83\) cycles)

<table>
<thead>
<tr>
<th>Inhibin A</th>
<th>Oestradiol concentration (day 3, nmol/l)</th>
<th>Oestradiol concentration (day 3, nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(aspiration day)</td>
<td>(aspiration day)</td>
<td>(aspiration day)</td>
</tr>
<tr>
<td>Inhibin A (aspiration day)</td>
<td>(pg/ml)</td>
<td>(pg/ml)</td>
</tr>
<tr>
<td>No. Age of the woman (%)</td>
<td>Diameter of the leading follicle (mm)</td>
<td>Diameter of the leading follicle (mm)</td>
</tr>
<tr>
<td>(years)</td>
<td>Thickness of the endometrium (mm)</td>
<td>Thickness of the endometrium (mm)</td>
</tr>
<tr>
<td>No. Age of the woman (%)</td>
<td>Diameter of the leading follicle (mm)</td>
<td>Diameter of the leading follicle (mm)</td>
</tr>
<tr>
<td>(&lt;80% increase)</td>
<td>(&gt;80% increase)</td>
<td>(&gt;80% increase)</td>
</tr>
<tr>
<td>42</td>
<td>12 (10–15)</td>
<td>6 (5–7)</td>
</tr>
<tr>
<td>&gt;32 (25–37)</td>
<td>64 (38–80)</td>
<td>6 (5–8.9)</td>
</tr>
<tr>
<td>(&lt;80% increase)</td>
<td>(&lt;80% increase)</td>
<td>(&lt;80% increase)</td>
</tr>
<tr>
<td>41</td>
<td>32 (25–37)</td>
<td>64 (46–86)</td>
</tr>
</tbody>
</table>

Values are median and range.

Values within columns with the same superscripts were significantly different. \(P < 0.05\).

Timing of aspiration on IVM pregnancy rate

(Gonal-F\textsuperscript{R}, Serono, Geneva, Switzerland), human chorionic gonadotrophin (HCG) 0.5 IU/ml (Profasi; Serono) and heat inactivated serum (HIS) from the patient (10%) or human serum albumin (HSA) (0.5%). Oocytes were cultured singly in 25 µl drops of IVM medium under paraffin oil at 37°C in 5% CO\textsubscript{2} and humidified air for 28–36 h.

Oocytes were denuded with hyaluronidase (IVF Science, Göteborg, Sweden) and mechanical pipetting. Motile spermatozoa were prepared by either PureSperm\textsuperscript{TM} (Cryos, Aarhus, Denmark), gradient separation or by swim-up. For ICSI, denuded oocytes were placed individually into 5 µl drops of Sperm Prep\textsuperscript{R} medium (Medi-Cult) and 2 µl of sperm suspension was placed into a 10 µl drop of polyvinylpyrrolidone (PVP) (IVF Science). ICSI was performed on all metaphase II oocytes. The oocytes were then cultured in Falcon Petri dishes in 10 µl droplets of IVF medium (Medi-Cult) until day 2 or 3 after fertilization at which time suitable embryos were replaced into the women.

Ova were examined daily for evidence of germinal vesicle breakdown, polar body extrusion, and atretic changes and determined to be either prophase I (PI) if the germinal vesicle was present or metaphase II (MII) when the first polar body was present. Fertilization was defined as the presence of two pronuclei. Embryos were scored on a scale of 1–4 where types 1 and 2 (<10% fragmentation) were considered to be transferable. A maximum of two embryos was transferred.

Endometrial priming consisted of 17β-oestradiol started on the day of oocyte retrieval, and the women received 2 mg orally three times per day. Two days after aspiration, treatment with intravaginal progesterone suppositories was initiated and continued until the pregnancy test. Oestrogen and progesterone were continued if the pregnancy test was positive until 50 days gestation.

The study was approved by the local ethical committee. All couples participated in the study after oral and written consent were given.

### Ultrasound measurements

Follicular diameters were measured by the same observer during transvaginal ultrasound scanning using a 7.5 Mhz transvaginal transducer (B & K Medical, Gentofte, Denmark). The follicular diameter was calculated as the mean of the longest follicular axis and the axis perpendicular to it. The endometrial thickness was measured in a sagittal plane between hypeechoic inner borders of the endometrium.

### Assays

Oestradiol was measured by radioimmunoassay, and interassay coefficients of variation were 10% for values <0.1 nmol/l, 8% for values between 0.2–1 nmol/l, 6% for values between 1 and 2 nmol/l and 5% for values >2 nmol/l. Inhibin A was measured in duplicate in double antibody enzyme immunometric assays (100 ml samples) using monoclonal antibodies raised against β\textsubscript{A} subunit in combination with a labelled antibody raised against the inhibin α-subunit as previously described (Groome et al., 1996). The detection limit of inhibin A was 7 pg/ml and the interassay coefficients of variation were 19.0% for values <14.9 pg/ml and 13.9% for values <31 pg/ml. Recombinant inhibin A was used for the standard curve.

### Statistical methods

Statistical analyses were done by Fisher’s exact test or \(\chi^2\) test. Because none of the hormone variables displayed a normal distribution, the non-parametric Mann–Whitney \(U\) test was used to analyse differences between unpaired data. Values were considered statistically significant when \(P < 0.05\).

### Results

In total 532 oocytes were aspirated and 388 were cumulus enclosed and could be used for IVM (73%). Fertilization with
ICSI was performed in 234 oocytes and fertilization with two pronuclei (2PN) was obtained in 180 (77%). In total, 156 cleaved (87%) and 125 were used for transfer. Eleven singleton gestations with a live fetus were obtained, giving a pregnancy rate per aspiration of 12.6% (11/87), a pregnancy rate per transfer of 17.4% (11/63) and an implantation rate of 8.8% (11/125). Nine healthy children have been born, and two pregnancies underwent abortion in the eighth to ninth gestational weeks.

Retrospectively, in 85 cycles the oestradiol concentration was obtained on day 3 and on the day of aspiration. An increase of 100% in the concentration of oestradiol was obtained on day 3 and on the day of aspiration. An additional weeks.

(11/125). Nine healthy children have been born, and two pregnancies underwent abortion in the eighth to ninth gestational weeks.

Retrospectively, in 85 cycles the oestradiol concentration was obtained on day 3 and on the day of aspiration. An increase of 100% in the concentration of oestradiol was observed in 57 cycles and an increase <100% was seen in 28 cycles. Significantly more pregnancies were obtained in cycles with an oestradiol increase (11/57, 19% per aspiration, 24% per transfer) compared with cycles without an increase (0/28, 0% and 0% respectively). No difference between maturation, fertilization or cleavage rate was observed (Table I).

Retrospectively, in 83 cycles the inhibin A concentration was obtained on day 3 and at the day of aspiration. In 42 of these cycles a detected oestradiol increase at least 100% plus a detected increase in inhibin A concentration of at least 80% were observed on the day of aspiration compared to day 3. If the value of inhibin A was <7 pg/ml on day 3 (n = 39), an increase to 13 pg/ml on the day of aspiration was required. In this group, 10 pregnancies were obtained after transfer in 30 cycles, giving a pregnancy rate of 24% per aspiration and 33% per transfer. In the group without an increase in inhibin A, no pregnancies were observed (Table II).

There were no statistical differences in age, day of aspiration as percentage of follicular phase, thickness of the endometrium or base values of oestradiol and inhibin A on day 3 in the two groups. The dominant follicle was significantly bigger in the group with a detected increase in oestradiol and inhibin on the day of aspiration ($P = 0.03$) (Table III).

No difference was seen in maturation, fertilization, cleavage or pregnancy rates between oocytes obtained after the leading follicle was $\geq 12$ mm in diameter compared with oocytes obtained before the leading follicle was 12 mm in diameter (Table IV), although a significantly thicker endometrium and a higher concentration of both oestradiol and inhibin A were found in cycles where the leading follicle was $\geq 12$ mm compared with cycles where the leading follicle was $< 12$ mm on the day of aspiration (Table V). Nevertheless, there was no difference between these groups in the number of cycles with a detected increase in both oestradiol and inhibin A were found (Table V).

### Safety

No severe adverse effects such as infection, bleeding or abdominal pain referred to as gynaecological were observed in this study. No extrauterine pregnancies were obtained.

### Discussion

Timing of aspiration may be crucial in IVM. By monitoring the size of the follicles and timing the aspiration to the day after a follicle of at least 10 mm diameter and a concomitant proliferative endometrium of at least 5 mm thickness could be demonstrated by transvaginal ultrasound, we were able to obtain a clinical pregnancy rate of 13% per aspiration and 17% per transfer in 87 consecutive cycles. If a concomitant increase in oestradiol could be detected on the day of aspiration compared to day 3, significantly more pregnancies were observed compared to cycles without an increase in oestradiol. Further elevation of the pregnancy rate was obtained if aspiration was performed after a detected increase in both oestradiol and inhibin A was demonstrated.

The maturation, fertilization and cleavage rates did not differ between the groups and this observation is not surprising. We know from previous studies that it is possible to mature oocytes *in vitro* and subsequently obtain fertilization and cleavage. The values in our study correspond with those in previous studies using immature oocytes from unstimulated women. Pregnancy rates, however, have been disappointingly low: 2% when up to three embryos are transferred (Barnes *et al*., 1996; Russell *et al*., 1997; Trounson *et al*., 1998). Some centres have transferred more embryos resulting in higher pregnancy rates (Cha and Chiang, 1998), but the implantation rate of embryos derived from in-vitro matured oocytes has been very low compared with the results in the present study. It has been questioned whether inadequate or incomplete cytoplasmic maturation of oocytes leading to developmental incompetence of embryos contributes to the high incidence of pregnancy failure in previous studies.

Cytoplasmic maturation is closely linked to all processes that prepare the oocyte for fertilization and embryo development. There is evidence from bovine studies that competent oocytes could originate from early atretic follicles (Sirard *et al*., 1998). If a similar mechanism is operational in humans, reduced follicular growth and incipient atresia may be advantageous before oocyte collection for IVM. This is supported by an earlier study (Gougeon and Testart, 1986) in which incipient stages of atresia may have been analogous to the incipient stages of oocyte maturation, possibly involving granulosa

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### Table IV. The maturation, fertilization and cleavage rates of oocytes obtained after a leading follicle of $\geq 12$ mm was seen compared to cycles where the leading follicle was $< 12$ mm ($n = 83$ cycles)

<table>
<thead>
<tr>
<th>Leading follicle</th>
<th>No. aspirations</th>
<th>No. oocytes</th>
<th>For IVM (%)</th>
<th>MII (%)</th>
<th>2PN (%)</th>
<th>Cleavage (%)</th>
<th>No. pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt; 12$ mm</td>
<td>41</td>
<td>248</td>
<td>175 (71)</td>
<td>104 (59)</td>
<td>81 (78)</td>
<td>75 (93)</td>
<td>5</td>
</tr>
<tr>
<td>$\geq 12$ mm</td>
<td>42</td>
<td>260</td>
<td>198 (76)</td>
<td>124 (62)</td>
<td>91 (73)</td>
<td>76 (84)</td>
<td>5</td>
</tr>
</tbody>
</table>

IVM = in-vitro maturation; 2PN = two pronuclei; MII = metaphase II.
Timing of aspiration on IVM pregnancy rate

Evidence in the literature regarding timing of aspiration before IVM in humans is conflicting. According to Russell, (1998), a decreased number of oocytes with decreased maturation rate and fertilization rate will be retrieved in follicular phase when the dominant follicle has exceeded 14 mm in diameter, and recently, Cobo et al. (1999) have found that once selection of the leading follicle has occurred, the developmental potential of the remaining oocytes is impaired. The explanation for these findings could be a negative effect of the selection process. Whitacre et al. (1998) did not find any influence of cycle phase on maturation rate, and recently Thornton et al. (1999) obtained pregnancies from human oocytes retrieved in unstimulated cycles during mid-cycle aspiration of immature oocytes. These investigators raised some doubts with regard to the significance of early atresia of the non-dominant follicles in unstimulated cycles.

The influence of FSH priming is controversial. While Wynn et al. (1998) pointed out that FSH priming increased the maturation rate, other studies (Trounson et al., 1998; Mikkelsen et al., 1999) were not able to detect any positive influence of FSH on oocyte in-vitro maturation rate, on fertilization rate or on pregnancy rate. This difference may be due to different timing of aspiration. A small dose of gonadotrophins to normally cyclic patients may increase the yield of immature oocytes collected, and this appears to be most effective if oocyte collection is delayed until oestrogen concentrations have begun to fall (Barnes, 1997) with a concomitant decrease in the follicular growth.

In the present study we intended oocyte collection to coincide with selection of the dominant follicle. It has been considered that the dominant follicle can be recognized by ultrasound when the diameter has reached 10 mm (Pache et al., 1990; Fauser and van Heusden, 1997).

Growth and development of the dominant follicle are correlated with secretion of both oestradiol (van Dessel et al., 1995) and inhibin A into the circulation. The increase in serum concentrations of inhibin A has been found to parallel that of oestradiol but with a delay of 1–2 days (Schipper et al., 1998; Lahlou et al., 1999). In humans a linear relationship between follicular fluid concentration of inhibin A and follicular size and maturation has been reported; therefore the increase in serum of inhibin A seems to give evidence of growth of the dominant follicle. In the present study pregnancies were only obtained in cycles where an increase in both oestradiol and inhibin A was observed, whereas none was obtained in cycles without a detected increase in these hormones. This indicates an improved cytoplasmic maturation of oocytes obtained from an advanced stage in folliculogenesis. The appearance of measurable inhibin A can be seen as a marker for a follicle having matured at least to the late follicular stage. The concentration of inhibin A may give information about the stage of follicular maturation on the day of the blood test and could contribute to the selection of the dominant follicle in combination with ovarian ultrasound measuring the size of the cell dissociation from the oocyte and accompanying loss of controlling factors. Furthermore, oocytes in atretic follicles may often be found to have progressed further than meiotic prophase I (Anderson et al., 1997).

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Table V: Clinical variables in the cycles (n = 83) with a leading follicle ≥ 12 mm diameter compared to those with a leading follicle < 12 mm diameter on the day of aspiration

<table>
<thead>
<tr>
<th>Diameter of the leading follicle (mm)</th>
<th>&lt;12 mm</th>
<th>≥12 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of aspiration (day 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness of the endometrium (mm)</td>
<td>5.8 (5–7.9)</td>
<td>6.5 (5.5–9.9)</td>
</tr>
<tr>
<td>Oestradiol concentration (nmol/l)</td>
<td>0.11 (0.04–0.43)</td>
<td>0.15 (0.04–0.37)</td>
</tr>
<tr>
<td>Inhibin A concentration (pg/ml)</td>
<td>0.25 (0.09–0.97)</td>
<td>0.37 (0.07–1.20)</td>
</tr>
<tr>
<td>Increase in oestradiol (%)</td>
<td>13 (&lt;7–20)</td>
<td>17 (&lt;7–15)</td>
</tr>
<tr>
<td>Increase in inhibin A (%)</td>
<td>18 (6–24)</td>
<td>24 (17–57)</td>
</tr>
<tr>
<td>Values are median and range.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p &lt; 0.05, Mann–Whitney.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Values are median and range.

**p** < 0.05, Mann–Whitney.
folicles. It is questionable, however, whether measuring inhibin A offers superior predictive powers of selection compared to oestradiol measurement. The high interassay variation for inhibin A concentrations (<14.9 pg/ml) is well known and in practice this means that measurement of inhibin A may be less useful in the prediction of aspiration compared with oestradiol.

Besides gonadotrophins that can improve the developmental competence of in-vitro matured oocytes by acting on the granulosa cells (Mason et al., 1994) and oestradiol that can influence directly the quality of the maturing oocyte (Tesarak and Mendoza, 1995), our medium contained serum from the patient obtained on the day of aspiration. This means that other hormones and growth factors may have played a role in the process of IVM. Studies in cattle suggest that inhibin A may play an important role during the final stages of oogenesis. Recent reports have indicated that inhibin A as well as activin may influence the cytoplasmic maturation, so these hormones or their combination in the serum may have significantly improved the embryonic developmental competence (Alak et al., 1998).

Follicle size and serum concentration of oestradiol have long been used as indicators of oocyte maturity in ovulation induction and conventional IVF. Both of these parameters are suggestive of the fertilization potential of oocytes in IVF cycles. We found in the present study that the same parameters may play a role in decision-making before aspiration in IVM. Furthermore, monitoring the concentration of inhibin A was shown to be of great value for assessing the optimal time for oocyte retrieval as a clinical pregnancy rate of 24% per aspiration was obtained in 41 cycles with a detected increase in both hormone values. These data may have some relevance with respect to improving clinical procedures to obtain immature oocytes which are fully developmentally competent. This will be followed up in a prospective study.

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References


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