Inhibition by human embryos of mouse granulosa cell progesterone production: development of a sensitive bioassay

A. Dhawan1,2, M.-C. Leveille3,4 and B.C. Vanderhyden1,2,4,5

1Ottawa Regional Cancer Centre, Ottawa, Ontario, 2Department of Cellular and Molecular Medicine, University of Ottawa, 3Fertility Center, Ottawa Hospital and 4Department of Obstetrics and Gynecology, University of Ottawa, Canada

5To whom correspondence should be addressed at: Ottawa Regional Cancer Centre, Cancer Research Laboratories, 501 Smyth Road, Ottawa, Ontario, Canada, K1H 8L6

Reproduction technologies could be improved by the development of methods to evaluate oocyte or embryo quality in a non-invasive, quantitative manner. Since human embryos secrete a factor that inhibits granulosa cell progesterone production, an interspecies bioassay was established to investigate whether the presence of this progesterone-inhibitory factor (PIF) in human embryo-conditioned (HEC) media is related to the health and developmental capacity of the embryos. Oocytes were microsurgically removed from oocyte–cumulus complexes isolated from superovulated mouse ovaries, and the ooyctectomized complexes were cultured in HEC media in the presence of follicle stimulating hormone and testosterone. Progesterone accumulation in the media was determined by radioimmunoassay. Despite the potential limitations of very small volumes of HEC media to evaluate, and the need to freeze these media at the source, the bioassay was able to detect PIF activity in HEC media. Most embryos produced PIF activity, but the degree of inhibition was not correlated with the ability of oocytes to be fertilized, nor with embryo morphology or ability to cleave and develop after transfer. These results demonstrate that secretion of PIF by human embryos can be measured by this bioassay and that human PIF can inhibit murine granulosa cell steroidogenesis; however, PIF activity is not correlated with human embryo quality or developmental competence.

Key words: bioassay/cumulus granulosa cells/embryo quality/progesterone-inhibitory factor/steroidogenesis

Introduction

The identification of variables that are linked with in-vitro fertilization (IVF) success would be beneficial in predicting pregnancy outcome and for enabling modification of IVF strategies to maximize odds for success. It is important to select the most viable oocytes/embryos (i.e. those most likely to develop into healthy fetuses and reach term) for transfer and to keep the transfer number small, to increase pregnancy rates and decrease the risk of multiple gestations with consequent obstetrical morbidity. The most successful methods of assessing oocyte and embryo quality in an IVF setting, without compromising their health, would ideally be non-invasive.

Non-invasive methods of assessment—both qualitative and quantitative—have been used to investigate potential predictive variables in follicular fluid, cumulus cells and embryos. Investigators have attempted to associate biochemical markers found in follicular fluid, i.e. proteoglycans (Suchanek et al., 1994; Eriksen et al., 1997), steroid hormones (Kreiner et al., 1987; Chui et al., 1997) and c-kit ligand (Smikle et al., 1998), with oocyte/embryo quality, fertilizability and pregnancy outcome. The predictive value of looking directly at cumulus or oocyte morphology as a gauge for reproductive success has been reported (Testart et al., 1983; Veeck, 1988; Mahadevan and Fleetham, 1990; Van den Bergh et al., 1995); however, others have found no predictive value (Balaban et al., 1998; Rattanachaiyanont et al., 1999), bringing into question the efficacy of using this widespread ‘predictive’ parameter in IVF centres.

Markers for embryo assessment have also been investigated for their ability to predict IVF success. Positive correlations have been found between morphological scoring of embryos and pregnancy outcome (Shulman et al., 1993; Visser and Fourie, 1993). Biochemical markers have also shown promise in prediction of pregnancy: O2 consumption (Magnusson et al., 1986), pyruvate uptake (Leese et al., 1986; Hardy et al., 1989; Conaghan et al., 1993), glucose uptake (Hardy et al., 1989), immunosuppressive activity (Jones et al., 1992) and embryo-derived platelet-activating factor (O’Neill et al., 1987).

The discovery of factors secreted by the oocytes of various mammals (e.g. mice, rats, pigs) raised the possibility of using these molecules as markers for oocyte quality and embryo developmental capacity. Oocytes secrete a factor that is essential for cumulus expansion (cumulus expansion enabling factor, CEEF); cumulus cells cultured in the absence of oocytes will not produce hyaluronic acid or undergo expansion (Buccione et al., 1990; Salustri et al., 1990). Oocyte-secreted factors have also been shown to promote the proliferation of granulosa cells (Vanderhyden et al., 1992; Lanuza et al., 1998) and to regulate steroid hormone production by granulosa cells (Vanderhyden et al., 1993). Of particular interest has been the observation that a progesterone-inhibitory factor (PIF) is secreted by human zygotes (Seifer et al., 1996). In mice, PIF has been found to be constitutively secreted throughout oocyte development (Vanderhyden and Macdonald, 1998) and at least up to the zygote stage (Vanderhyden et al., 1993). The presence of this factor during oocyte development suggests that it plays an important role in the prevention of premature luteinization of the follicle. A defect in the oocyte that impeded PIF
secretion would affect follicular growth and reduce the health and developmental capacity of the resulting embryo.

In the present study, we attempted to develop a novel and non-invasive method to detect and measure the amount of PIF activity in human embryo-conditioned media and to correlate this activity with embryo health and developmental capacity.

Materials and methods

Collection of spent media

Human embryo-conditioned media (HEC) samples were obtained from the IVF programme of the Ottawa Hospital. The IVF procedure involved the collection of oocyte–cumulus cell complexes (OCC) from follicular aspirates and culturing them singly for 4 h in 1 ml human tubal fluid media (HTF; Meditech IST; Montreal, Quebec, Canada) containing 0.5% w/v bovine serum albumin (BSA; Bayer, Kankakee, IL, USA) at 37°C in an atmosphere of 5% O₂, 5% CO₂ and 90% N₂. After the initial 4 h incubation, the OCC were inseminated singly in 50 µl drops of HTF–BSA with ~6250 motile spermatozoa, for approximately 16–18 h, whereupon the OCC were stripped of their cumulus cells and assessed for fertilization by the presence of two pronuclei. Fertilized oocytes were then individually cultured for an additional 48 h in 20 µl drops of HTF–BSA.

For oocytes fertilized by intracytoplasmic sperm injection (ICSI), OCC were collected from follicular aspirates and cultured for 2–3 h in the same conditions as for IVF. The oocytes were subsequently denuded of cumulus cells, and their maturity was assessed immediately under a stereomicroscope. The oocytes were further cultured in 20 µl drops of HTF–BSA for 1–2 h, after which ICSI was performed on all MII oocytes (as assessed by absence of germinal vesicle and presence of polar body) using motile spermatozoa isolated by density gradient centrifugation. Injected oocytes were assessed 18 h after injection for fertilization and were individually cultured for 42 h before transfer.

The use of a composite embryo score, consisting of the product of cell number and morphological quality, has been reported previously as a tool to select high-grade embryos (Joesbury et al., 1998) and, therefore, a similar composite score was used in this study. The morphological quality and stage of embryo growth were assessed qualitatively at 42 h after insemination. Embryo morphology was graded with a score of 1–5 according to blastomere quality and level of fragmentation: grade 5 = regular blastomeres and no anucleated fragments; grade 4 = regular blastomeres and <25% fragmentation; grade 3 = regular or irregular blastomeres and <25% fragmentation; grade 2 = regular or irregular blastomeres and 25–50% fragmentation; and grade 1 = regular or irregular blastomeres and >50% fragmentation (Rattanachaiyanont et al., 1999). Points for embryo cell number (growth) were given according to the number of cells observed up to the 4-cell stage (e.g. 2-cell embryo was given a score of 2). Embryos with a cell number of five were given 3 points and those embryos with a number of cells greater than six were given scores of 2. Embryos of good quality were considered to be those that had achieved at least a 4-cell stage with a morphological quality of at least grade 3 (e.g. score = 12). The minimum score that could be given was 1, and the maximum was 20.

Embryos were transferred 48 h post-retrieval and were selected for transfer on the basis of the combined embryo score of morphological quality and growth. The remaining spent (HEC) media from both oocytes (failed fertilization, n = 44) and embryos (n = 47) were immediately frozen at −20°C until use in experiments. In total, 91 samples of HEC media from individually cultured oocytes and embryos, resulting from either IVF (n = 38) or ICSI (n = 53) procedures, were evaluated. These samples were generated from the oocytes of 25 different patients. For all experiments, control media refer to fresh aliquots of HTF–BSA media frozen for approximately the same length of time as the HEC media before use in the experiments.

Collection of mouse oocyte–cumulus cell complexes and denuded oocytes

Animals were maintained and handled according to the Guidelines for the Care and Use of Animals established by the Canadian Council on Animal Care. Ovaries were isolated from 22- to 26-day-old (C57BL/6×Balb/c) F1 mice 44–48 h after injection with 5 IU pregnant mares’ serum gonadotrophin (PMSG; Folligon; Intervet Canada Inc., Whitby, ON, Canada). The superovulation of the ovaries yielded approximately 40–50 OCC per mouse. On the day of the experiment, the ovaries were removed from the animals and the antral follicles were punctured with 25-gauge needles in Waymouth MB752/1 culture medium (WAY) supplemented with 75 mg/ml penicillin-G, 50 mg/ml streptomycin, 25 mg/ml sodium pyruvate (all from Sigma Chemical Co., Milwaukee, WI, USA), and 5% fetal bovine serum (FBS; Hyclone Laboratories Inc., Logan, UT, USA). After isolation, the complexes were washed twice in fresh WAY–FBS. For positive controls for cumulus expansion experiments, denuded mouse oocytes were used. OCC were isolated from the antral follicles of PMSG-treated mice, and oocytes were denuded by repeated pipetting with a Pasteur pipette. Denuded oocytes were then washed twice in WAY–FBS. To produce oocytectomized cumulus complexes (OOX), oocytes were microsurgically removed from OCC using a micromanipulation apparatus and the procedure for oocytectomy as described previously (Buccione et al., 1990).

Culture of intact and oocytectomized complexes

Cumulus expansion

To assess the presence of CEEF in HEC media, OCC and OOX were cultured in 50 µl drops of frozen–thawed HTF (control) or HEC medium supplemented with 5% FBS and follicle stimulating hormone (FSH) (NIADDK-oFSH-19; 300 ng/ml) under washed mineral oil (Fisher Scientific, Fair Lawn, NJ, USA) in 35 mm Petri dishes (Falcon; Becton Dickinson and Co., Franklin Lakes, NJ, USA). Since the embryos were cultured in only 20 µl, the HEC media samples were diluted 2.5-fold in HTF to generate the 50 µl drops. To confirm the ability of OOX complexes to expand in the presence of CEEF, some drops of OOX included 10 denuded mouse oocytes. The complexes were cultured for 16 h at 37°C in 5% CO₂:5% O₂:90% N₂ and then assessed for the degree of cumulus expansion using a subjective scoring system that ranges from 0 (no response) to +4 (maximal expansion) as described previously (Buccione et al., 1990).

Steroid regulation

Since HEC media samples were frozen at the source, initial experiments were conducted to ensure retention of the ability of granulosa cells to produce progesterone in frozen–thawed media. Two OCC or OOX were cultured in 50 µl drops of HTF–FBS per well of 96-well plates (Costar; Corning Costar Corp., Cambridge, MA, USA). In addition, limited volumes of HEC media (20 µl per embryo) were available; thus, the ability to detect differences between OCC and OOX needed to be established by determining appropriate culture conditions. To determine the minimum culture volume at which PIF activity could be detected, two OCC or OOX were cultured in a volume of 20 to 350 µl of HTF–FBS per well of 96-well plates. To determine the optimal number of complexes to be cultured in the appropriate volume of medium, a range of one to 10 OCC and OOX were cultured in 100 µl of HTF–FBS media. For all experiments
and OOX were stimulated with FSH in 50 µl of pooled HEC media to near-maximal levels (150 ng/ml FSH and 500 nmol/l testosterone) to promote progesterone production. After 48 h, the culture media were collected and stored at −20°C until assayed for progesterone using a radio-immunoassay that has been described and validated for direct measurement (Daniel and Armstrong, 1984). In the initial experiments, the optimal culture conditions for the bioassay were determined to be the culture of two complexes in 100 µl of culture medium; therefore, these conditions were then used to bioassay the HEC media for progesterone inhibitory activity.

To assess the effects of HEC medium on progesterone production, as well as the effectiveness of HEC media following 5-fold dilution, two OOX were added to either 100 µl of pooled HEC media (same patient) or 20 µl of pooled HEC media diluted to 100 µl with HTF. These media were supplemented with 5% FBS, 150 ng/ml FSH and 500 nmol/l testosterone and the complexes were cultured for 48 h. The culture media were then stored at −20°C until assayed for progesterone. Progesterone accumulation in the HEC media were compared with the progesterone production by two OOX cultured in frozen–thawed control (HTF) media under identical conditions, and the degree of HEC media-induced changes in progesterone production were compared with the ability of the oocytes/embryos that were cultured in that medium to be fertilized, to undergo cleavage, to develop into morphologically healthy embryos, and to establish pregnancy.

Statistical analysis
All experiments were performed at least three times, with different pools of ovaries and at least two replicates per pool. When comparing multiple groups, data were expressed as mean ± SEM, and statistical comparison was made using analysis of variance with the Newman–Keuls test for multiple comparisons. Statistical comparisons of two groups were made using unpaired, two-tailed t-tests for normal distributions and non-parametric, Mann–Whitney t-tests for non-Gaussian distributions (e.g. non-fertilized versus fertilized, non-pregnancy versus pregnancy). Statistical significance was inferred at P < 0.05.

Results
Assaying for cumulus expansion-enabling factor in HEC media
To test for the presence of CEEF in HEC media, mouse OCC and OOX were stimulated with FSH in 50 µl drops of HTF or HEC media (Table I). As expected, OCC underwent cumulus expansion to near-maximal levels (+3 to +4), whereas OOX cultured in control HTF did not expand. Although OOX could expand in the presence of denuded oocytes, HEC media failed to support expansion of the OOX complexes. With this inability to detect CEEF in HEC media under these conditions, the remaining experiments focused on the detection of PIP in the HEC media.

Potential limitations on the assessment of HEC media
The first potential limitation in testing the HEC media was the necessity to freeze the media, which could impact on the ability of the media to support steroidogenesis. OCC produced significantly less progesterone compared with OOX complexes for both media groups (Figure 1; P < 0.05). There was no significant difference in progesterone production between the fresh and frozen–thawed media groups. Therefore, freezing the HTF medium did not impair its ability to support the steroidogenesis of murine cumulus complexes.

The second potential limitation involved in the assessment of the HEC media was culture volume. Due to the small volumes of HEC media (20 µl) used to culture each embryo, it was necessary to determine the minimal culture volume that could be used to avoid significant dilution of the HEC, and without affecting the health and, as a consequence, steroidogenic ability of OCC and OOX. In order to identify the minimum culture volume, two OCC or OOX were cultured in a range of volumes from 20 µl to 350 µl HTF media for 48 h (Figure 2A). Concurrent with the increase in culture volume was a dramatic (5-fold) increase in FSH + testosterone-stimulated progesterone production by both OCC and OOX (OCC, from 1.7 ± 0.3 to 8.3 ± 1.1 ng/complex; OOX, from 5.3 ± 1.2 to 26.3 ± 6.7 ng/complex). However, for each culture volume, OOX produced significantly greater amounts of progesterone than OCC (P < 0.05). Since cultures in 100 µl generated a large difference between OCC and OOX in their progesterone production with a relatively low level of variability, this volume was determined to be the optimal culture volume.

Table I. Cumulus expansion of follicle stimulating hormone-stimulated intact (OCC) and oocytectomized (OOX) cumulus complexes cultured in the absence or presence of human embryo-conditioned (HEC) media

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of complexes assessed</th>
<th>No. of HEC media samples</th>
<th>Degree of expansion (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCC</td>
<td>50</td>
<td>0</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>OOX</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OOX + DO</td>
<td>25</td>
<td>0</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>OOX + HEC</td>
<td>65</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

DO = denuded oocyte.
Figure 3. Progesterone production by OOX complexes cultured in 100 µl pooled HEC (e.g. A^100) media or in 20 µl of the pooled HEC media diluted to 100 µl (e.g. A^20). HEC media were obtained from three different patients (A, B and C). OCC and OOX cultured in HTF were used as controls. Complexes were cultured for 48 h in the presence of 5% FBS, 150 ng/ml FSH and 500 nmol/l testosterone. *Significantly different from OCC (P < 0.05).

Effect of HEC media on murine cumulus cell progesterone production

To test the effects of dilution of HEC media on murine cumulus cell progesterone production, two OOX were cultured in a total volume of either pooled (100 µl) (same patient) or diluted (20 µl of the same pooled samples diluted to 100 µl with HTF media) HEC media for 48 h. Control OOX, cultured in 100 µl HTF, produced significantly (P < 0.05) more progesterone per complex than OCC, which were 20% of OOX control. For three patients (A, B, C), HEC media inhibited progesterone production from 22 to 99%, with the greatest inhibitory activity exhibited by diluted HEC media upon murine OOX, the remaining HEC media samples were tested for their ability to regulate progesterone production by OOX.

Bioassay of HEC media and relationship between PIF activity and embryo developmental capacity

There was tremendous variability in progesterone regulatory activity among HEC media samples (n = 91; Figures 4 and 5), with the effects ranging from stimulation (182% of progesterone production by OOX complexes in control medium), to the greatest inhibition at 11% of control. However, the vast majority (92%) of samples elicited some degree of inhibition, whereas only four samples showed stimulation. No correlation (r = 0.013) was found between the level of progesterone production by OOX in HEC media from cultured embryos and the embryo score for the embryos cultured in that medium (Figure 4). The embryo scores, derived from...
Regulation of granulosa cell steroidogenesis

Figure 4. Progesterone-inhibitory factor (PIF) activity in HEC media of fertilized embryos presented as a percentage of progesterone production relative to control (OOX in HTF medium). OOX complexes were cultured in the presence of 150 ng/ml FSH and 500 nmol/l testosterone for 48 h. Progesterone production in individual HEC media samples are plotted with respect to each embryo’s score. The line indicates linear regression analysis ($r = -0.013$).

Assessment of embryo morphology and growth, ranged from a minimum score of 1 to a maximum of 20. The degree of variability for progesterone inhibition by HEC media was similar for all embryo scores, suggesting that PIF activity does not change with improved embryo quality. A comparison of embryo morphology or cell number independently with PIF activity found no correlation, indicating that PIF activity is not influenced by embryo morphology or growth (data not shown).

Figure 5A shows the progesterone production (% of control) of OOX complexes cultured in HEC media grouped according to the absence or presence of normal fertilization of the oocytes. Using a non-parametric (Mann–Whitney) t-test, no significant difference was found between the ability of media from non-fertilized ($n = 44$) and fertilized oocytes ($n = 47$) to inhibit cumulus cell progesterone production (median: 39.5 and 51% of control levels respectively). Analysis of the data from oocytes fertilized by IVF versus ICSI showed no difference when each of these subgroups was compared with the group of non-fertilized oocytes; however, ICSI-fertilized oocytes tended to be less effective in suppressing progesterone production than IVF-fertilized oocytes (median = 60 versus 35.5% of control respectively).

Of the total number of embryos ($n = 37$) that were transferred into the 25 patients, seven were among the transferred embryos that resulted in five pregnancies (one from ICSI; four from IVF). A comparison of HEC media samples from embryos that were transferred in non-pregnant cycles versus pregnant cycles revealed no significant difference in their ability to inhibit murine cumulus cell progesterone production (median: 58.5 and 32% respectively; Figure 5B).

Figure 5. (A) Progesterone production, as a percentage of control (OOX in HTF medium), by OOX cultured in HEC media from non-fertilized and fertilized (two pronuclei) oocytes. (B) Comparison of progesterone production by OOX complexes cultured in HEC media samples from embryos that were transferred in non-pregnant versus pregnant cycles. Complexes were treated with 150 ng/ml FSH and 500 nmol/l testosterone for 48 h. Each datum point is the accumulation of progesterone by OOX complexes cultured in HEC media samples from individually cultured embryos. The lines represent the medians of all values in that group.

Discussion

The experiments reported here identify the optimal conditions for a bioassay that can be used to determine quantitatively the activity of the progesterone-inhibitory factor produced by human oocytes and embryos. Despite the potential restrictive limitations of very small volumes of culture medium to evaluate, and the need to freeze this medium at the source, the bioassay could be used to measure the progesterone
suppressive activity in human oocyte/embryo-conditioned media. Most embryos produced the factor(s) that inhibited progesterone production, but the degree of inhibition did not correlate with the ability of the oocytes to be fertilized, nor with embryo morphology or their ability to cleave or develop after transfer.

Human oocyte/embryo culture media failed to enable cumulus expansion of murine OOX cumulus complexes. CEEF has been shown to be secreted by meiotically competent oocytes (Vanderhyden et al., 1990) of various mammalian species (e.g. mice, rats, pigs) (Buccione et al., 1990; Vanderhyden, 1993), and the factor appears to act across species; i.e. pig and rat oocytes secrete a factor that enables the expansion of mouse OOX complexes (Vanderhyden, 1993). Murine zygotes have been shown to secrete CEEF, although the degree of expansion that they elicit is below maximal (Vanderhyden et al., 1990). The absence of any response from HEC media upon OOX complex expansion may be explained by an insufficient amount of CEEF, since only one embryo conditioned 20 μl of media, whereas previous studies have used two oocytes/embryos to condition each μl of media (Vanderhyden et al., 1990). It is also possible that the human CEEF, if one exists, cannot enable the expansion of murine cumulus cells. Although this assay system was unable to detect CEEF, future identification of the biochemical nature of CEEF may permit its detection in HEC media, with the potential to develop assays of greater sensitivity.

In both fresh and frozen–thawed media, OOX complexes produced significantly greater amounts of progesterone than OCC, confirming our previous findings (Vanderhyden et al., 1993; Vanderhyden and Tonary, 1995) that murine oocytes secrete a factor which inhibits progesterone production by granulosa cells. Freezing the culture media (for up to 8 weeks) had no effect on the ability of the media to support progesterone production by murine OOX complexes.

The optimal bioassay conditions were determined by identifying the minimum culture volume and minimum number of OOX complexes that could be used while retaining the ability to produce progesterone at levels that could be influenced by the presence of oocytes or oocyte-conditioned media. For volume experiments, a 17-fold increase in culture volume resulted in a 5-fold increase in progesterone accumulation by both OCC and OOX. All culture volumes enabled a significant difference between OCC and OOX in their progesterone production, although a large difference, along with a low variability, was observed for 100 μl, identifying that volume as optimal for the bioassay. An increase in the number of cumulus complexes per well resulted in an overall decrease in progesterone production per complex. A similar trend was demonstrated (Bar-Ami and Khoury, 1994) when changing the cell-plating density of cumulus cells in culture, and progesterone production was found to decrease with a concurrent increase in cell-plating density. These results suggest the possible presence of a progesterone concentration threshold at which negative feedback regulation of progesterone production by cumulus granulosa cells occurs. The particular culture conditions chosen as optimal (two complexes, 100 μl) were due to the relatively low variability observed in combination with a large difference between OOX and OCC progesterone production, thus allowing for a large range within which progesterone inhibition could be observed and a minimal dilution of HEC media.

In these studies, PIF activity was detected in HEC media, suggesting that human oocytes and embryos are capable of secreting a factor that can regulate murine granulosa cell steroidogenesis. We have shown previously that PIF is secreted by human zygotes; zygotes co-cultured on monolayers of human granulosa cells suppressed granulosa cell progesterone and oestradiol production (Seifer et al., 1996), suggesting a human germ cell influence on granulosa cell activity. Mouse oocytes secrete PIF throughout follicular development, from mid-growth to full-grown stages as well as ovulated oocytes and zygotes (Vanderhyden and Macdonald, 1998), although later stages of embryonic development have not yet been examined. It is possible that secretion of PIF by human embryos is the continuation of its constitutive production during oocyte development.

The observations that mouse (Vanderhyden et al., 1993), pig (Coskun et al., 1995), Xenopus (Sretarsuga and Wallace, 1997) and human oocytes (Seifer et al., 1996, and this study) can regulate granulosa steroid hormone production may explain, at least in part, the ‘directing’ influence of the oocyte upon follicular development that has been reported previously (Wallace, 1983; Hubbard and Erickson, 1988). Secretion of PIF by oocytes likely inhibits premature luteinization of granulosa cells since the presence of PIF inhibits both progesterone production and the induction of luteinizing hormone receptors (Eppig et al., 1997). Since follicular fluid contains a factor that prevents luteinization of granulosa cells (Ledwitz-Rigby and Rigby, 1979), measurements of this factor in follicular fluid may be a viable alternative to the bioassay described in this study. It is possible that defects in oocyte or embryo function could impair the secretion of PIF, thus contributing to conditions that potentially cause infertility, such as unruptured follicle syndrome or unexplained anovulatory cycles.

Since ovarian production of progesterone increases during the preimplantation stages of human embryo development, the role of embryo-secreted PIF is unclear. Studies have shown that high concentrations of progesterone adversely affect embryo quality and IVF pregnancy rates. Premature luteinization with increased progesterone concentrations negatively affects oocytes, leading to a reduction in their fertilizability (Schoolcraft et al., 1991). A subtle rise in serum progesterone concentrations during the follicular phase has also been associated with reduced implantation rates (Harada et al., 1995). Embryonic secretion of progesterone inhibitory factors may be a mechanism to maintain at least local progesterone concentrations at reduced levels.

Regardless of its possible functions, the demonstration of PIF secretion by human embryos enabled the establishment of a non-invasive bioassay with the aim to correlate the amount of factor secreted by embryos to their developmental capacity. The variability in the degree to which HEC media inhibited progesterone production by cumulus cells suggests that individual oocytes/embryos produce different amounts of PIF or
levels of PIF activity. Unfortunately, no correlation was found between the level of PIF and human oocyte fertilizability or embryo developmental capacity. The observation that ICSI-fertilized oocytes tend to produce less PIF activity than IVF-fertilized oocytes suggests a possible difference between ICSI- and IVF-fertilized oocytes in this aspect of embryo function, and warrants further investigation on a larger scale, particularly with regard to the value of these differences in predicting the ability of the embryo to establish pregnancies.

Further studies aimed at elucidating the identity and mechanism of action of oocyte- and embryo-secreted factors will be of tremendous benefit in developing accurate, non-invasive methods to evaluate oocyte and embryo maturity, health and developmental potential. Identification of such factors will enable the quantitative assessment of embryo quality and may improve embryo culture conditions or the receptivity of the uterus for implantation.

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A.Dhawan, M.-C.Léveillé and B.C.Vanderhyden


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