Pretreatment with follicle stimulating hormone promotes the numbers of human oocytes reaching metaphase II by in-vitro maturation

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The objective of this study was to investigate the effect of follicle stimulating hormone (FSH) priming on the in-vitro maturation (IVM) of human oocytes from healthy ovaries using a chemically defined culture system. Seventeen patients donating oocytes for research received a truncated course of 600 IU FSH over 5 days and a further control group of nine patients received no FSH treatment. Mid-follicular phase cumulus-enclosed oocytes (n = 160) were aspirated from follicles ≤ 4 mm diameter under transvaginal ultrasound guidance and were cultured for 48 h in micro-drops of medium containing 10 mIU/ml FSH and 100 mIU/ml human chorionic gonadotrophin (HCG). The results demonstrated that human oocytes will efficiently undergo IVM under serum-free conditions. After mild FSH stimulation, a greater number of cumulus-enclosed oocytes was collected, and following culture, a lower rate of degeneration was observed. Significantly more oocytes completed nuclear maturation to metaphase II following FSH stimulation (71.1 versus 43.5%). In conclusion, a truncated course of FSH stimulation in vitro improved the oocyte maturation rate in vitro, giving a mean of 4.8 ± 0.7 metaphase II oocytes per patient compared with only 2.1 ± 0.7 from control patients, thus yielding more mature oocytes for future IVF treatment.

Key words: FSH/granulosa cells/human/in-vitro maturation/oocyte

Introduction

Following the first demonstration by Pincus and Enzmann (1935) that germinal vesicle stage oocytes from the rabbit could mature spontaneously in vitro, the process of in-vitro maturation (IVM) has become such an efficient method of producing oocytes for in-vitro fertilization (IVF) that it is now a commercial proposition in farm animals (Trounson et al., 1994a). The potential benefits of developing an effective IVM programme as an alternative clinical strategy to conventional IVF are many: it would not only substantially reduce both the costs of drug treatment and wastage of immature eggs collected during standard IVF (16.3% of oocytes; Van Steirteghem et al., 1993), but could also lessen the risks of hyperstimulation syndrome and, more controversially, ovarian carcinoma. Additionally, IVM may provide a valuable model for investigating the causes of meiotic aberrations and aneuploidies which are remarkably common in mature human oocytes (Gras et al., 1992; Delhanty et al., 1997). Finally, IVM might open the door to oocyte cryopreservation if freeze storage of germinal vesicle stage oocytes avoids the problem of damaging the meiotic spindle of metaphase II oocytes (Toth et al., 1994). In view of all these issues, IVM deserves rigorous experimental evaluation to determine whether it is practicable in reproductive medicine.

The first demonstration of IVM in the human was reported by Edwards (1965), and appeared a theoretically attractive method for obtaining oocytes for IFV. However, hormonal stimulation proved to be more effective. Although pregnancies had been recorded using IVM oocytes following conventional FSH stimulation with human chorionic gonadotrophin (HCG) administration (Veeck et al., 1983), it was not until 1991 that the first clinical pregnancy was achieved using oocytes collected from unstimulated ovaries and matured in vitro (Cha et al., 1991). IVM has since been applied to mature oocytes collected at the germinal vesicle stage following conventional IVF with HCG administered in vivo (Nagy et al., 1996) or without HCG (Jaroudi et al., 1997; Liu et al., 1997).

These reports suggest that the development of IVM–IVF is feasible in humans, but the technology requires further evaluation as success rates in establishing clinical pregnancies are unacceptably low at 1–2% (Cha et al., 1991; Trounson et al., 1994b; Russell et al., 1997). Possible explanations of these disappointing results include: the poor quality of oocytes recovered from some of the patients (e.g. with polycystic ovaries), many of whom had previously failed to conceive with conventional IVF; suboptimal culture conditions; and the timing of embryo development in relation to the physiological stage and degree of development of the endometrium. In none of these studies, however, were the ovaries exposed to gonadotrophin stimulation prior to oocyte collection. IVM studies in rhesus monkeys (Schramm and Bavister, 1994) have indicated that mild ovarian stimulation with follicle stimulating hormone (FSH) prior to oocyte retrieval improved both the proportion of oocytes that were meiotically competent and success during subsequent embryonic development. It has not been clear whether a truncated course of FSH stimulation might improve the development of human oocytes matured in vitro.

The three major studies of human IVM (Cha et al., 1991; Trounson et al., 1994b; Russell et al., 1997) have used a serum or follicular fluid based culture medium. In contrast, some studies in animals (Eppig et al., 1992; Serta et al., 1995) and a short report in humans (Kennedy and Donahue, 1969) have
demonstrated that serum-free conditions can support oocyte maturation in vitro. Indeed, such conditions carry important advantages in terms of safety and quality control of culture media. The potential risks of zona hardening during culture in the absence of serum, as indicated in animal studies (Schroeder et al., 1990; Schramm and Bavister, 1996), and the requirement therefore for intracytoplasmic sperm injection (ICSI) to effect fertilization, requires further investigation.

The objectives of this investigation were to determine the effect of a truncated course of FSH pretreatment on the numbers of oocytes recovered and their progress to metaphase II. Accordingly, we have recruited patients of proven fertility and tested the efficacy of mild FSH stimulation in vitro on oocyte developmental potential in vitro using a serum-free culture system.

Materials and methods
Patients who were of proven fertility and under 37 years of age (range 23–36) and were referred to the Leeds General Infirmary for sterilization were recruited to the study and consented under a protocol approved by the local ethics committee. Women with >10 follicles per ovary as assessed by ultrasonography were considered to have polycystic ovaries (Adams et al., 1985) and those who had a body mass index >27 were excluded from the study. A pilot study to test the efficiency of recovering and culturing oocytes after FSH stimulation was performed on eight volunteers (group 1). The study was subsequently extended to include a larger group of comparable volunteers who were randomized to receive either FSH stimulation (group 2) or no stimulation (control, group 3). Groups 1 and 2 received a truncated course of recombinant FSH (Gonal-F, Serono, Herts, UK) by subcutaneous injection as follows: 300 IU on day 2, 150 IU on day 4 and 150 IU on day 6 of the cycle; day 1 being designated as the onset of menstrual bleeding. Peripheral venous blood samples were collected by venepuncture and the serum concentrations of FSH, luteinizing hormone (LH), oestradiol and progesterone were recorded on days 2, 7 and 12 of the cycle. Serum FSH and LH were measured using an ACS180 autoreader (Chiron, UK), progesterone concentrations were determined using an Autoelida autoreader (Vallac, Finland) and the oestradiol concentrations were measured using in-house radioimmunoassay after prior extraction with organic solvent. Calibration curves were generated in tubes containing the residue from an appropriate volume of the solvent (Atkinson et al., 1996). On days 2, 7 and 12, the endometrial thickness was estimated by transvaginal ultrasound.

Oocytes were retrieved under general anaesthesia on day 7 of the cycle prior to sterilization. A 16 gauge double lumen needle (DCIS/16G/Clarendon, Casmed, Surrey, UK) was used to recover the oocytes using a reduced aspiration pressure compared with conventional IVF of only 80 mmHg, as described by Trounson et al. (1994b). Other aspects of the collection technique were similar to those used in routine IVF (Horne et al., 1996). The diameters of all follicles ≥4 mm were measured in two planes and recorded. Individual follicles were aspirated and flushed three times with HEPES-buffered Earle’s balanced salt solution (EBSS, Gibco, Paisley, UK) containing 93.3 U/ml penicillin G and 38.2 U/ml streptomycin sulphate and supplemented with preservative free heparin 20 U/ml (Monoheparin, CP Pharmaceuticals, Wrexham, UK) into pre-warmed, sterile, 12 ml Falcon tubes (Becton Dickson Labware, Lincoln Park, USA). Unless otherwise stated, all culture media and additives were obtained from Sigma Chemical Company (Poole, Dorset, UK). Each follicular aspirate was examined under the binocular microscope and recovered cumulus–oocyte complexes (COC) were transferred to pre-warmed, 96-well tissue culture plates (Costar, Cambridge, UK) containing 20 μl microdrops of medium per well under 50 μl mineral oil. The culture medium consisted of bicarbonate-buffered minimum essential medium with Earle’s salts with the following additives: 0.1% human serum albumin (Zenalb, BPL, Elstree, Herts, UK), 0.47 mM sodium pyruvate, 93.3 IU/ml penicillin G, 38.2 U/ml streptomycin sulphate, 5 μg/ml human transferrin, 4.8 ng/ml sodium selenite, 10 ng/ml human recombinant insulin, 100 ng/ml Long R3 IGF-1 (Groopez Pty Ltd, Adelaide, Australia), 3 mM L-glutamine, 10 mM FSH (Gonal-F) and 100 mU/ml HCG (Boehringer, East Sussex, UK). The cultures were incubated at 37°C in 5% CO2 in air.

Time 0 was designated as the time that the oocytes were placed into culture. At 0, 24 and 48 h, the COC were examined and graded according to the degree of cumulus–oocyte coverage: ≤3 layers of granulosa cells (GC) score 0; 3 ≤3 <10 layers of GC score 1; ≥10 layers of GC score 2; and cumulus expansion: tight, dense corona cells score 0; moderate expansion score 1; fully expanded score 2 (see Figure 1). Where possible, the meiotic status of the oocyte was noted prior to culture, however, in the majority of cases the dense cumulus cells made accurate assessment impossible. After 48 h in culture, the oocytes were mechanically stripped of cumulus cells by repeated pipetting and the meiotic status of each oocyte was recorded. The cumulus cells from individual COC were stored in 20 μl of culture medium at –70°C for later determination of cell number. Measurement of total DNA content was performed with the fluorochrome dye, Hoechst 33258 (bisbenzimidazole; Sigma) using a ‘Microfluor’ automatic microplate fluorescence reader (Dynatech, Billingshurst, West Sussex, UK) as described by Boland and G OSDEN (1994). A standard curve was set up using calf thymus DNA with values ranging from 40 to 8000 ng. To calculate approximate cumulus cell numbers an estimate of 6 pg of DNA per cell was used.

In a second study to investigate the timing of oocyte maturation 56 COC were collected from eight FSH treated volunteers. The stimulation protocol, collection technique and culture system used were the same as described above. After 20 h in culture, the oocytes were mechanically denuded of cumulus cells in the culture droplet and oocyte maturity assessed under the dissecting microscope. Following examination, each oocyte together with the original cumulus cells from the COC were co-cultured and their meiotic status re-examined at 28, 36, 44 and finally 52 h of culture.

The effect of FSH pretreatment on follicular development, serum hormone concentrations, endometrial thickness and DNA content of COC were compared using Student’s t-test. To normalize the distribution, oestradiol data were transformed by log10 prior to analysis. The effect of FSH pretreatment on the meiotic competence of individual oocytes in vitro was analysed using χ2 and Fisher’s exact tests. The relationship between follicular size, cumulus mass and oocyte maturation were further analysed using Spearman’s rank correlation.

Initial analysis indicated no significant difference between the FSH-treated groups (1 and 2) for the following parameters: patient age (30.3 ± 0.9 versus 28.4 ± 1.9 years respectively); gravidity (3.0 ± 0.6 versus 3.6 ± 0.4 respectively); number of follicles aspirated (10.8 ± 1.4 versus 12.6 ± 1.2); the serum oestradiol on day 7 (1270 ± 386 versus 883±315 pmol/l) or the maturation rate (84.6 versus 64.0%). These two data sets were therefore combined to enable comparison of FSH treatment (groups 1 + 2) with the control group.

There were no differences in age or number of previous pregnancies between the FSH treated group (1 + 2), the control group 3 and those patients entered into the timing study (FSH treated: mean age 29.3 ± 1.1 years and gravida 3.3 ± 0.4; control: mean age 28.7 ± 1.1 years).
Results

Five days of treatment with Gonal-F was effective in stimulating the development of significantly more follicles of ≥4 mm diameter than in the ovaries of control patients (see Table I). There was no significant difference in the mean diameter of FSH stimulated follicles (range 4–16 mm) compared with controls (range 5–11 mm).

More oocytes were collected from FSH-treated patients than controls (7.47 ± 1.2 versus 5.2 ± 1.3), but this was not significant. Fewer degenerating oocytes, both at the time of oocyte recovery (1.6 versus 13.0%, P < 0.01) and after 48 h of culture (5.5 versus 17.4%, P < 0.05) were observed from patients treated with FSH (groups 1 + 2) compared with group 3.

Table I. Effect of follicle stimulating hormone (FSH) treatment on ovarian follicular development and function. The values shown are mean ± SEM for nine control and 17 FSH-treated patients

<table>
<thead>
<tr>
<th></th>
<th>Control n = 9</th>
<th>FSH-treated n = 17</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean follicle diameter (mm) at collection ± SEM</td>
<td>8.0 ± 0.5</td>
<td>8.9 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Mean number of follicles aspirated ± SEM</td>
<td>7.6 ± 1.2</td>
<td>11.7 ± 1.2</td>
<td>0.039</td>
</tr>
<tr>
<td>Mean number of oocytes collected ± SEM</td>
<td>5.2 ± 1.3</td>
<td>7.5 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Mean serum oestradiol (pmol/l) on day 7 ± SEM</td>
<td>154 ± 17</td>
<td>1049 ± 241</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean serum progesterone (nmol/l) on day 12 ± SEM</td>
<td>2.4 ± 0.4</td>
<td>2.9 ± 0.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significantly different.
Table II. Effect of FSH priming on oocyte maturation and cumulus mass DNA content after 48 h in culture.
The values shown are mean ± SEM for the number of oocytes in each individual category.

<table>
<thead>
<tr>
<th>Category</th>
<th>Control group</th>
<th>DNA (ng/COC)</th>
<th>FSH-treated</th>
<th>DNA (ng/COC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of oocytes (% total)</td>
<td>Mean ± SEM</td>
<td>Number of oocytes (% total)</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Degenerate</td>
<td>8 (17.4)</td>
<td>16.8 ± 5.9</td>
<td>6 (5.3)</td>
<td>17.2 ± 1.3</td>
</tr>
<tr>
<td>GV</td>
<td>5 (10.9)</td>
<td>5.4 ± 3.2</td>
<td>12 (10.5)</td>
<td>20.1 ± 3.7</td>
</tr>
<tr>
<td>MI</td>
<td>13 (28.3)</td>
<td>13.9 ± 3.9</td>
<td>15 (13.2)</td>
<td>21.1 ± 3.5</td>
</tr>
<tr>
<td>MII</td>
<td>20 (43.5)</td>
<td>8.5 ± 2.4</td>
<td>81 (71.1)</td>
<td>21.1 ± 2.1</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>11.1 ± 1.9</td>
<td>114</td>
<td>21.3 ± 1.6</td>
</tr>
</tbody>
</table>

NS = not significantly different; COC = cumulus–oocyte complex; GV = germinal vesicle; MI = metaphase I; MII = metaphase II.

Figure 2. The effect of FSH pretreatment on oocyte outcome with IVM. *P < 0.02. metaphase II; metaphase I; germinal vesicle stage; degenerate.

3 patients who received no FSH. After 48 h in culture a similar percentage of oocytes from FSH-treated compared with non-treated patients had initiated meiosis (84.2 versus 71.7%). However, a significantly greater percentage of oocytes from the FSH-treated patients completed meiotic maturation to metaphase II in vitro (71.1 versus 43.5%, P < 0.05; see Table II). This gave rise to significantly more metaphase II oocytes per patient in the FSH-treated group (FSH-treated; mean 4.8 ± 0.7 versus controls; 2.1 ± 0.7, P < 0.02: see Figure 2).

Within the FSH-treated group, successful oocyte maturation was positively correlated with follicle size (P < 0.01) and degree of cumulus expansion after 48 h of culture (P < 0.05). Irrespective of the FSH pretreatment protocol, the minimum follicular size supporting oocyte maturation was 5 mm. The meiotic potential of oocytes from all groups tended to be impaired in those with little or no covering of cumulus cells (score 0) at the time of egg collection, but this was not statistically significant. A significantly higher DNA content was found in the COC of the FSH-treated group (P < 0.01), approximating to 3550 cumulus cells per COC compared with only 1850 in controls.

The mean serum oestradiol concentrations on the day of egg collection (cycle day 7) were significantly higher after FSH treatment (Table I). In contrast, serum progesterone concentrations remained low but comparable in the FSH-treated patients and the unstimulated controls and the endometrial thickness on day 12 was not significantly different following FSH treatment (FSH-treated: 4.9 ± 0.7 mm versus control: 5.5 ± 1.2 mm).

In the second study to investigate the timing of maturation, the mean number of eggs collected per patient (7.0 ± 1.3) did not differ significantly from the initial study. At the removal of cumulus cells after 20 h of culture, 15 oocytes (26.8%) were degenerate, 12 (21.4%) were at the germinal vesicle-stage, 27 (48.2%) had commenced meiosis and were classified metaphase I and only two oocytes (3.7%) were already at metaphase II (equivalent to 7.7% of the oocytes that ultimately reached metaphase II). At 28, 36 and 44 h, the number of metaphase II oocytes increased to 14 (53.8%), 20 (76.9%), and 25 (96.2%) respectively. By 52 h, a total of 26 oocytes had reached meiotic maturity. The percentages of oocytes both initiating meiosis (60.7%) and reaching metaphase II (46.6%) were significantly less than observed in oocytes from the FSH stimulated patients in groups 1 + 2 (P < 0.01), whilst the percentage undergoing degeneration (32.1%) was significantly increased (P < 0.01). Changes in number of oocytes at each meiotic state for time point are shown in Figure 3.
Discussion

This is the first report of in-vitro maturation of human oocytes from healthy ovaries using a serum-free culture system. We have demonstrated by a randomized trial that a truncated course of stimulation with FSH prior to oocyte collection improves developmental potential as assessed by nuclear maturation to metaphase II. This finding is consistent with physiological expectations and agrees with data obtained in rhesus monkeys (Schramm and Bavister, 1994). What is more, a chemically defined medium containing salts, an energy source, pure hormones and serum albumin supported oocyte maturation, with a high percentage (71.1%) of oocytes progressing to metaphase II. In other clinical studies, the media used contained follicular fluid or serum (Cha et al., 1991; Trounson et al., 1994b; Russell et al., 1997), both of which are undesirable on grounds of safety and quality control. The serum-free medium used in the current studies was based on a formula previously shown to support preovulatory concentrations of oestradiol production by porcine granulosa cells (Picton et al., 1994, 1998).

In our study, the resumption of meiosis occurred in a similar percentage of oocytes from both the FSH-treated (84.2%) and control (71.8%) groups, during subsequent culture 39.4% of those arrested before reaching metaphase II in the controls, compared with only 15.6% in the FSH-treated group. Similar results have been demonstrated in animal studies (Sorensen and Wasserman, 1976; Wickramasinghe et al., 1991); however, the underlying cause of the meiotic arrest in human oocytes has yet to be investigated. Our data suggest that in-vivo human oocytes only acquire full meiotic competence late in follicular development after exposure to above threshold concentrations of FSH (Brown, 1978) and prior to the pre-ovulatory luteinizing hormone surge. As the FSH receptor gene is expressed exclusively in granulosa cells (Hillier et al., 1994), the effects of FSH administration must be mediated through the cumulus cells by promoting cumulus cell steroid production, oocyte RNA and protein synthesis and possibly inhibiting granulosa cell apoptosis (McGee et al., 1997). These observations are also consistent with a requirement for protein synthesis to continue until the time of germinal vesicle breakdown in human oocytes (Schultz et al., 1988). Further studies are required to determine the earliest stages of follicular development at which oocytes can be harvested for efficient and effective IVM.

We have shown that pretreatment with FSH doubled the numbers of cumulus cells collected with the oocyte. It is possible that the presence of increased cumulus cell numbers in culture is a contributory factor, as they may actively synthesize maturation promoting factors, energy substrates and hormones and probably metabolize any toxins.

The second part of our study was to establish the time course of meiotic maturation in vitro in order to define the optimum time for fertilization. Oocytes were collected from volunteers following treatment with recombinant FSH. After 20 h in culture, 69.6% of oocytes were immature, 26.8% were degenerate and only two (7.7%) oocytes had extruded a polar body. One of these oocytes was noted to have been in the germinal vesicle stage prior to culture, as it was devoid of cumulus at collection, a fact which may in itself have stimulated premature development (Dekel and Piontkewitz, 1991). The number of oocytes that reached metaphase II was significantly less than seen with oocytes from similar patients also receiving FSH in the first part of our study (46.6 versus 71.1% respectively). This reflects suboptimal culture conditions due to increased handling of the oocytes during stripping and repeated examination thereafter. The high percentage of degenerate oocytes cannot be attributed to the experimental conditions, as nine of the 18 degenerate oocytes were recovered from one patient. Furthermore, 15 of these oocytes were degenerate the time of the first inspection. The reduced maturation potential may also be due to the suboptimal pH associated with gas and temperature changes during oocyte handling and inspection. The use of mineral oil will have helped to minimize this effect. Additionally, oocytes were mechanically separated from their surrounding cumulus cells after only 20 h of culture so disrupting oocyte–somatic cell contacts earlier than would be expected in vivo. Co-culture of oocytes with their cumulus cells was not sufficient to overcome this effect, so highlighting the importance of the cumulus–oocyte interactions in the attainment of meiotic maturation.

Oocytes were seen to reach metaphase II throughout the course of the 52 h experiment, and >96% of the maturing oocytes had done so within 44 h of the start of culture. Indeed, 77–96% of oocytes had reached metaphase II between 36 and 44 h from the start of culture in a similar time frame to that seen in vivo (Larsen, 1993). These observations agree with the data of Edwards (1965) and Trounson et al. (1994b) on human oocytes.

Whilst a chemically defined culture system allows the opportunity to study energy metabolism and endocrine function of the oocyte, prolonged culture in the absence of serum may have undesirable side-effects on the oocyte such as premature cortical granule release, which leads to hardening of the zona pellucida which would reduce the fertilization potential for IVF (Green, 1997). Additionally, zonal hardening has been shown to reduce the rate of blastocyst hatching in the rhesus monkey from 77% in serum based medium to only 25% in serum free culture (Schramm and Bavister, 1996). Zona hardening has never been quantified in human IVM oocytes; however, approaches to minimize its impact, such as fertilization with ICSI followed by assisted hatching, have been successfully applied to human IVM oocytes (Barnes et al., 1995). Ultimately, it may prove necessary to perform both ICSI and assisted hatching for all IVM oocytes and embryos. Or, alternatively, with adaptations to the culture medium such as the addition of the serum derived antioxidant fetuin, we might be able to overcome the potential problem of zona hardening (Schroeder et al., 1990).

The high percentage of oocyte nuclear maturation and low incidence of degeneration presented in the randomized study do not, however, necessarily imply that the metaphase II cells produced are fertile. A previous report has demonstrated that 18% of morphologically normal human oocytes matured in vitro had gross meiotic aberrations (Racowsky and Kaufman, 1992). While such aneuploidies in IVM oocytes would reduce
successful fertilization and subsequent early embryo development, they have also been shown to occur at a similar rate during oocyte maturation in vivo (20%) and at even higher rates after gonadotrophic stimulation of mature oocytes for IVF or gamete intra-Fallopian transfer (GIFT) stimulated cycles (Gras et al., 1992; Munné et al., 1993; Delhanty et al., 1997). Furthermore, it remains unclear if oocytes matured in vitro have accumulated the entire payload of RNA and proteins required to support early post-fertilization embryonic development. It is essential, therefore, to extend the present work and test the cytotogenetic integrity of IVM oocytes by IVF with or without ICSI and to monitor the resultant embryos' normal development and ploidy before we attempt embryo replacement in a clinical trial.

It is clear from our results that even with FSH treatment endometrial thickness and luteal function were inadequate to support implantation (Navot et al., 1989; Shapiro et al., 1993).

It is anticipated that adequate endometrial preparation through steroid priming and luteal supplementation as demonstrated Russell et al. (1997) would overcome this shortfall and would be essential to support the establishment and maintenance of a pregnancy.

In conclusion, the truncated FSH stimulation regimen used in this study provides the first evidence that mild stimulation with FSH is desirable to improve both human oocyte yield and maturational competence. This experimental protocol still represents a significant economic saving compared with conventional IVF therapy and is consistent with the widely felt need to minimize ovarian stimulation and avoid hyperstimulation. Indeed, this culture system may have a role in conventional IVF by maximizing the numbers of oocytes available for ICSI. The immature oocytes collected (16.3%; Van Steirteghem et al., 1993) can undergo IVM prior to fertilization and this has already given rise to clinical pregnancies (Veeck et al., 1983; Nagy et al., 1996; Liu et al., 1997). Furthermore, the major disadvantages of using a serum based medium can be avoided, as we have demonstrated human oocytes will undergo a high rate of maturation in a chemically defined culture system. Finally, we believe that despite the poor pregnancy rates seen in human trials so far, the record of success in laboratory and farm animals is extremely encouraging and with the benefits of FSH pretreatment IVM–IVF can in principle provide an attractive alternative to conventional IVF.

Acknowledgements

We gratefully acknowledge Angela Mklanda for assistance in carrying out the DNA assays. We thank Serono UK for the gift of Gonal-F, the National Health Service for a Research and Development grant to Roger Gosden and Well-Being for providing a project grant to support a research assistant.

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Received on January 21, 1998; accepted on July 31, 1998