

EFFECTS OF r-FSH ADDITION, IN CULTURED MEDIA, ON OOCYTE IN-VITRO MATURATION

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ABSTRACT

Objectives : To study the effect of addition of low dose of recombinant follicle stimulating hormone(r-FSH) in culture media on oocyte maturation, fertilization, cleavage and pregnancy rates in women with polycystic ovaries.

Material and Methods : In a randomized study of 100 women in an IVF program, 50 patients with 674 germinal vesicle (GV) oocytes were allocated to study the effect of low concentration dose of r-FSH (0.0 and 0.075) supplemented in culture media on oocytes maturation, fertilization, cleavage, and pregnancy rate.

Results : Oocyte maturation rate significantly ($P < 0.05$) increased from 47% at 0.0 (control) to 81% at 0.075 IU/ml of r-FSH concentration, respectively. Fertilization, cleavage, and clinical pregnancy rates showed a similar trend and significantly increased from 45% to 83% and from 32% to 80% and from 0 to 17% at the two r-FSH concentrations, respectively.

Conclusions : The results suggested that r-FSH supplementation in the culture media concentration of 0.075 IU was optimum in the present study and improved GV maturation, fertilization, cleavage and pregnancy rates.

Key words : Oocytes, IVM, ICSI, fertilization, pregnancy

INTRODUCTION

Since the delivery of the first three children originating from in-vitro-matured oocytes obtained from ovarian biopsy specimens⁽¹⁾, research in in-vitro maturation of human oocytes has shown significant progress and provided hope for certain groups of patients who have infertility problems. Human oocytes recovered from immature follicles, following retrieval can resume and complete meiosis

in-vitro when cultured in media supplemented with r-FHS and hCG^(2,3,4,5). Published reports had shown that in-vitro matured oocytes could be fertilized, and result in pregnancy^(6,2) and birth of healthy babies^(1,7). Despite the clinical utility of IVM in the field of human reproduction, its pregnancy and birth rates remain low compared to in-vitro matured oocytes. Nevertheless, IVM remained a low cost procedure and an optimum solution to some causes of infertility in certain groups of patients. Women with polycystic ovary syndrome have typical symptoms of abnormal

endocrine parameters, hyperandrogenism, anovulation, numerous antral follicles in the ovary on ultrasound scan and infertility⁽²⁾. In PCOs patients the dominance of a particular follicle fails to occur and the cohort of the numerous growing follicle accumulate in the cortex⁽⁸⁾. These patients are extremely sensitive to exogenous gonadotropin when used for assisted reproduction and may develop ovarian hyper-stimulation, deep vein thrombosis⁽⁹⁾ and ovarian cancer resulting from prolonged use of fertility drugs. There is now increasing interest to avoid these risks, by retrieving oocytes using minimal or no gonadotropins stimulation and then maturing them in-vitro in culture medium containing recombinant FSH, LH or hCG. When oocytes are matured in-vitro, r-FSH or urinary gonadotrophins are added to the culture media to improve oocyte maturation in various studies⁽¹⁰⁾. Recently both recombinant hCG and LH in addition to FSH were used to achieve oocyte maturation⁽⁴⁾, but without standardizations of the optimum concentrations which will yields the best results. This study aimed at assessing the effect of low dose r-FSH concentrations in culture media on oocyte maturation, fertilization and developmental competence.

MATERIALS & METHODS

Patients

Women with amenorrhea received oral contraceptive Marvelon® (Organon) once daily for 21-45 days to induce withdrawal bleeding at a specified time to program the cycle. A baseline vaginal ultrasound scan was performed for all women between day 1 and 2 of menstrual bleeding to ensure that no ovarian cysts were present. Ovarian stimulation with r-FSH 300 IU was given daily for five days and continued as necessary. Transvaginal ultrasound scans were repeated on either cycle day 8 and/or the day fo hCGH administration to exclude the

development of a dominant follicle. The size of all follicles on ultrasound scan had to be < 10 mm in diameter at every scan to proceed to oocyte retrieval, which was performed between days 8 and 10 of the cycle. All patients received 10000 IU of hCG 36 hours before oocytes retrieval.

Oocyte retrieval and IVF Procedure

Transvaginal ultrasound guided oocyte collection was performed using a specially designed 17-G single-lumen aspiration needle (Casmed, UK) with a reduced aspiration pressure of 7.5 kpa. Aspiration of all small follicles was performed under general anesthesia for all patients. Oocytes were collected in culture tubes containing warm Earl's balanced salt solution with 5000 IU/ml heparin. Immature oocytes were incubated in a culture dish containing 1 ml of 3M (Medicult) medium supplemented without or with 0.075 IU/ml r-FSH (Puregon, Organon) and 5.00 IU/ml hCG (Pregnyl, Organon) at 37°C in an atmosphere of 5% CO₂ and 95% air with high humidity. After culture, the maturity of the oocytes was determined under the stereo-microscope at 24 and 48 hours post collection. Oocytes were denuded of cumulus; maturity was determined by the presence of the first polar body. Suitable oocytes were injected with single spermatozoa by micromanipulation (Research Instrument, UK). Following ICSI, each oocyte was transferred into 1 ml of Medi-cult IVF medium in a tissue culture dish. Fertilization was assessed 18 hours after ICSI for the appearance of two distinct pronuclei and two polar bodies. Oocytes with two pronuclei were further cultured in Medi-cult IVF medium. Embryos were transferred on day 2 or 3 after ICSI.

Endometrial priming

For endometrial preparation, patients received estradiol valerate, Progynova (Schering Pharmaceutical, UK) starting on the day of oocyte retrieval, depending on the endometrial thickness on that day. If the endometrial thickness was <mm, a

6mg dose was given and if it was >4mm, a 6mg dose was given. Luteal support was provided by 100mg of progesterone (Gestone, Schering Pharmaceuticals, UK) once daily starting on the day of ICSI and continued, along with estradiol valerate until day 14 from the day of embryo transfer when a blood test for Beta hCG done to ascertain pregnancy. If there was pregnancy, the luteal support is continued till 12 weeks gestation.

The effect of FSH concentration in the culture media on in-vitro maturation of oocytes

For this study the patient group consisted of 50 women with PCO and they were all scheduled for ICSI. Patients were stimulated with 300 IU r-FSH (Purgeon, Organon, Holland). The aim was to study the influence of follicle stimulating hormone concentration on the maturation, fertilization, cleavage and pregnancy. Recombinant FSH was added in two concentrations: 0.00, and 0.075 IU/ml. This experiment was performed on patients producing more than 15 GV oocytes. Immediately after oocytes collection, the oocytes were equally aliquot in two groups of 5 oocytes each in two center-well dish containing 3ml Ham's F10. 0.075 IU/ml r-FSH concentration was added in one dish and none as control, respectively. Oocytes maturation were then assessed 30 hours after incubation.

RESULTS

Our data showed that 0.075 IU/ml provided higher maturation, fertilization and pregnancy rates than the control. Details regarding number of oocytes collected, maturation, fertilization and pregnancy rates after in vitro maturation are shown in table I. Recombinant FSH concentration had significantly ($p<0.05$) increased the rate of oocyte maturation from 47% at 0 IU to 81% at 0.075 IU/ml, respectively. Fertilization, cleavage, and clinical pregnancy rates showed a similar trend and significantly increased from 45% to 83%, from 32% to 80% and from 0 to

17% at the two concentrations, respectively. The 6 pregnancies resulting from oocytes cultured in media containing 0.075 IU/ml all ended in delivery of healthy children.

DISCUSSION

The present study showed that in-vitro matured oocytes from PCOS patients had the potential to undergo successful maturation, fertilization and the resultant embryos showed good developmental competence. Following the IVM procedure, embryo transfer culminated in clinical pregnancies and birth of healthy children. All the oocytes retrieved in this study were at the Germinal vesicle (GV) stage. The latter were defined as germinal vesicle stages (GV) which represent oocytes arrested at prophase of meiosis-I with prominent discernable germinal vesicle nucleus. There are various factors that effect oocytes in-vitro maturation; most important among these are the exposure of immature oocytes to gonadotrophin in culture media. Recombinant FSH, LH, and hCG⁽⁴⁾, and purified gonadotropin⁽¹¹⁾ were used to induce oocytes maturation in vitro. In this study the effect r-FSH addition to culture media on oocytes maturation, fertilization, cleavage, and pregnancy rates was investigated. In the present study in-vitro maturation rates of oocytes cultured in media containing 0.075 IU/ml was 81% which is higher when compared with 62%⁽¹²⁾, 67%⁽¹³⁾, 71%⁽⁶⁾, 61%⁽¹⁴⁾, 66%⁽¹⁵⁾, 58%⁽¹⁶⁾, and 55.9%⁽⁴⁾.

The variations in maturation rates between the present study and those mentioned above may be due to the composition of the culture medium used and proteins supplement. In the present study our optimum culture time (30h) was comparable with other studies⁽¹⁶⁾. Inadequacies of cultured media can not be ruled out as a possible cause for low IVM success⁽¹⁵⁾. There is evidence to suggest that culture media used for IVM adequately support nuclear maturation, but failed to produce oocytes with cytoplasmic maturation. While we used synthetic

serum supplement as a source of proteins in other studies fetal bovine serum (FBS) was used. FBS was considered more crucial for bovine oocyte maturation than human. In addition, our base medium was Hams F10 which was designed to meet the nutritional and maturational needs for human oocytes. Other reasons for the low maturation, fertilization and the developmental competence may be due to polycystic ovary syndrome as the main cause of infertility. Recent reports had shown that the low maturation rates in IVM can be improved by priming patients with gonadotrophins. Patient priming with r-FSH and hCG before retrieval may have contributed to the increase of the maturation rate in our study. Trounson and colleague (2001) reported a higher maturation rate (71%) in FSH treated women when compared with untreated women (44%). Follicle priming with r-FSH and hCG before oocyte retrieval had significantly increased the rate and speed of oocyte maturation⁽³⁾. It was found that 75% of oocytes recovered from superovulated and primed patients reached metaphase II after 30h of culture while the same percentage was reached after 42-45h of culture. Despite the relatively high maturation, fertilization and cleavage rates shown in the present study, our pregnancy rate remained relatively low (22.5%) compared to 27%⁽¹⁶⁾ and 40%⁽²⁾. The low pregnancy rate shown in IVM cases in general is partly attributed to abnormality during cytoplasmic maturation⁽⁵⁾. However pregnancy in other studies reached even higher rates (40%) following administration of 10000 IU hCG before immature oocyte retrieval^(2,17). In the present study, all patients were primed with 5000 IU hCG rather than 10000 IU to reduce the risk of OHSS, and this might have contributed to the low pregnancy rate. According to Chian et al. 1999, Son et al. (2002) and Hreinsson et al., (2003)^(2,18,4), hCG priming improved the percentage of oocytes achieving maturation and hastens the maturation process. The overall oocyte quality might be reduced among the oocytes retrieved from PCOS due to the high androgen level. Although

hCG priming improve i maturation rate of immature oocytes⁽¹⁷⁾, there is no evidence to suggest that FSH priming has a similar effect on pregnancy rates except for few exceptional cases⁽¹⁰⁾. In other studies, FSH priming made no difference to oocyte recovery, maturational and developmental potential, fertilization rate and pregnancy rates^(3,5). The low implantation and pregnancy rate in IVM may be due to asynchrony in the cytoplasmic and nuclear maturation in the oocytes⁽⁶⁾. According to Trounson et al., (2002), cytoplasmic protein necessary for the development of embryos can be produced only upon the oocytes completing cytoplasmic maturation⁽⁸⁾. It was also reported that oocytes from PCO show compromised developmental potential compared to regular cyclic patients. The use of gonadotrophin stimulation to produce multifollicular growth may adversely affect natural growth of the oocytes and results in poor pregnancy rates. Our data suggested that when oocytes were cultured in IVM media containing 0.075 IU r-FSH a comparable or even better maturation rates and developmental competence were obtained than results in many other recent reports. On the other hand, the results also showed that the above parameters were lower when the oocytes were incubated in media that do not contain r-FSH. In the present study, factors affecting immature oocyte maturation and developmental competence were not fully explored. There are many gaps that need to be bridged and other factors need to be closely investigated. The effect of growth hormone in culture media during oocyte maturation, chromosomal anomalies as well as the effect of anesthesia are both worthwhile to be investigated thoroughly.

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Table I : Effects of recombinant FSH addition on oocyte maturation, fertilization, embryo cleavage and clinical pregnancy.

Parameters	r-FSH concentrations (IU)	
	0.00	0.075
GV collected	225	219
Matured oocytes	105 (47%) ^a	178 (81%) ^b
Fertilized oocytes	47 (45%) ^a	147 (83%) ^b
Cleaved embryos	15 (3%) ^a	119 (80%) ^b
Transferred embryos	12 (2/ET)	55 (1.4 ET)
Clinical Pregnancy	0.00 ^a	6 (17%) ^b
No ET	6	39

N.B Data with similar superscripts letters are not significantly ($p>0.05$) different

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