The Effect of Growth Hormone on the Development of *In Vitro* Matured Unstimulated Human Oocytes

AREEPAN SOPHONSRITSUK, M.D.*,
KAMTHORN PRUKSANANONDA, M.D.*,
VICHUDA AHNONKITPANIT, M.Sc.*,
PRANEE NUMCHAISRIKA, B.Sc.*,
PRAMUAN VIRUTAMASEN, M.D.*
SOMCHAI SUWAJANAKORN, B.Sc., M.D.*,
WISAN SEREEPAPONG, M.D.*,
DOENTIP CHOMPURAT, B.Sc.*,
WISUT BOONKASEMSANTI, M.D.*,

Abstract

Objective : To investigate the effect of growth hormone on the development of *in vitro* matured unstimulated human oocytes.

Design : Randomized controlled study

Setting : Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn university

Material and Method : 108 germinal vesicle-stage oocytes were retrieved from 47 patients undergoing gynecologic surgery. They were aspirated either during gynecologic surgery or from excised ovaries. The oocytes were then cultured *in vitro* with or without growth hormone (1,000 ng/ml) in medium199 supplemented with sodium pyruvate, FSH, LH, antibiotic and synthetic serum. Incubation was done at 37 degree C with 5 per cent CO_2 in air and nuclear stage was assessed after 18, 42, 66 and 90 h of incubation.

Main outcome measure : Attainment of metaphase II and GVBD

Results : After *in vitro* culture, there were no significant differences in maturation and GVBD rate. 27 of 52 (51.9%) oocytes (GV) in growth hormone group matured to metaphase II compared with 25 of 53 (47.2%) GV in control group. GVBD rate for germinal vesicle-stage in growth hormone group was 76.9 per cent compared with 79.2 per cent in control group.

Conclusion : Culture of immature oocytes *in vitro* with growth hormone results in similar maturation rate as that without GH.

Key word : Growth Hormone, *In Vitro* Maturation, Immature Oocyte

**SOPHONSRITSUK A,
SUWAJANAKORN S, PRUKSANANONDA K, et al
J Med Assoc Thai 2002; 85: 907-914**

* Department of Obstetrics & Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Human oocytes usually become arrested in prophase I of meiosis during fetal life. Oocyte maturation, characterized by germinal vesicle breakdown (GVBD), formation of the first meiotic spindle (metaphase I), expulsion of the first polar body and arrest in metaphase of the second meiotic division (metaphase II) occurs in preovulatory follicles in response to the surge of gonadotropin and leads to an ovulated oocyte[1,2].

Standard protocols for assisted reproductive technology (ART) use exogenous gonadotropins to stimulate the growth of multiple follicles. There are many risks which are the consequences of using high levels of exogenous gonadotropins such as nausea and vomiting, ovarian hyperstimulation syndrome and increasing costs of ART[3].

The recovery of immature oocytes followed by *in vitro* maturation (IVM) of the oocytes could be developed as a new method for the ART program. *In vitro* maturation would avoid the need for gonadotropin stimulation of the ovaries, reducing costs and avoiding the risk of ovarian hyperstimulation syndrome. *In vitro* maturation can be used in patients with polycystic ovarian syndrome who are extremely sensitive to stimulation with gonadotropins and are at increased risk for the development of ovarian hyperstimulation syndrome[4].

Furthermore, the ability to mature oocytes *in vitro* would benefit pre-menopausal women who suffer from cancer and are likely to become sterile as a result of radio- or chemo-therapy. However, these *in vitro* matured oocytes demonstrate low rates of maturation. As a consequence, methods to optimize maturation and *in vitro* development conditions are being investigated; including the addition of gonadotropins and growth factor in culture media[5,6], coculture immature oocytes with granulosa cells[7]. The effect of gonadotropins on ovarian follicular function are thought to be mediated *via* the local production of steroid hormone and a wide variety of locally produced growth factors[8]. Granulosa and cumulus cells secrete a wide variety of growth factors that may either amplify or attenuate gonadotropin action in the ovary in a paracrine-autocrine manner.

The intraovarian GRF/growth hormone (GH)/IGF system is interesting for studying. *In vitro* studies demonstrated that GRF, GH, IGF accelerated maturation in cumulus-enclosed rat oocytes[2,9]. The positive effect of growth hormone has been shown in bovine oocytes as well. Oocytes matured in the presence of growth hormone showed an acceleration of nuclear maturation and a higher fertilization rate [10]. Therefore, the present study was conducted to investigate the effect of growth hormone on *in vitro* maturation of human unstimulated immature oocytes.

MATERIAL AND METHOD

Sources of oocytes and oocyte recovery

This study was approved by the Ethics Committee of Chulalongkorn hospital. The study was performed from May 1, 2001 to January 31, 2002. The inclusion criteria of the oocytes is the germinal vesicle stage oocytes which were collected from patients aged between 20 and 45 years, having regular menstruation, no hormonal treatment for at least 1 month and no ovarian pathology in the collected ovary. Germinal vesicle stage oocytes were collected from 2 sources. One source was from patients' ovaries during gynecologic operation. Another was from excised ovaries after oophorectomy. After oophorectomy, the ovaries were washed 3 times with physiologic saline and the visible antral follicles were aspirated with a 23- gauge needle to obtain the oocyte-cumulus complex. The follicular fluid was pooled into dish of T6 media supplemented with synthetic serum. Oocyte-cumulus masses were identified under a dissecting microscope.

The GV oocytes were cultured in one of two culture media following simple randomization. 1 in tissue culture medium 199 (Sigma Chemical Co., St. Louis, MO) supplemented with 10 per cent synthetic serum, 0.075 IU human menopausal gonadotropin/ml (Metrodin HP; Sereno, French Forest, New South Wales, Australia), 0.5 IU hCG/ml (Pregnyl; Organon), 0.05 mg/ml penicillin. 2 in the same culture media as 1, but 1,000 ng human somatropin/ml (Norditropin; Novo Nordisk, Denmark) was added in culture media. Each cumulus enclosed oocyte was cultured in one well of a four well dish at 37 degree C, in 5 per cent CO_2 in air atmosphere.

After incubation, oocytes were examined for maturation under inverted microscope at 18, 42, 66 and 90 hours after oocyte retrieval. The nucleus of GV oocyte was determined to be either metaphase I if the germinal vesicle was absent (Fig. 1) or metaphase II if the first polar body was present. (Fig. 2)

Statistical analysis

Percentages of germinal vesicle breakdown and maturation to metaphase II were compared be-

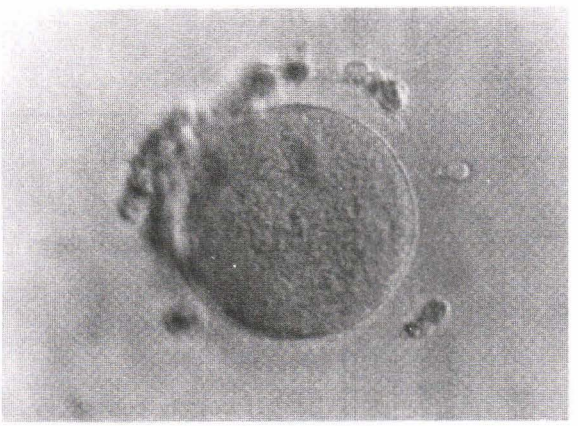

Fig. 1. Metaphase I human oocyte.

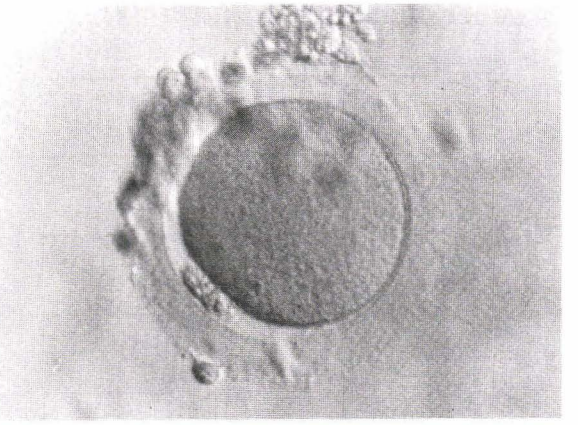

Fig. 2. Metaphase II human oocyte.

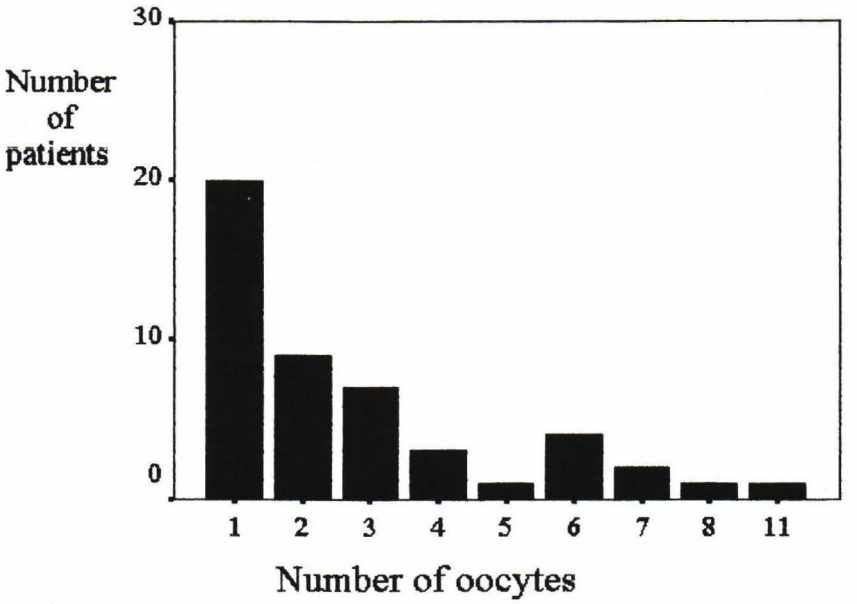

Fig. 3. Number of oocytes retrieved per one ovary.

tween groups with the pair *t*-test. Comparison of baseline characteristics of oocytes was performed using pair *t*-test and χ^2 test contingency. Statistical significance was defined as $p<0.05$.

RESULT

A total of 132 cumulus-oocyte complexes were retrieved from 47 patients, i.e. a mean (±SD) of 2.8±2.1 complexes per patient. Amount of oocytes retrieved per ovary is shown in Fig. 3. After being assessed under the inverted microscopy it revealed that 81.8 per cent of the oocytes were at the GV stage, i.e. a mean of 2.3 GV stage oocytes per patient. A total of 108 immature oocytes were collected, 19 (17.6%) oocytes from the patients' ovaries during gynecologic operation and 89 (82.4%) oocytes from excised ovaries. The immature oocytes were equally randomized in 2 groups; the first group was cultured in medium supplied with growth hormone, while the other was without growth hormone.

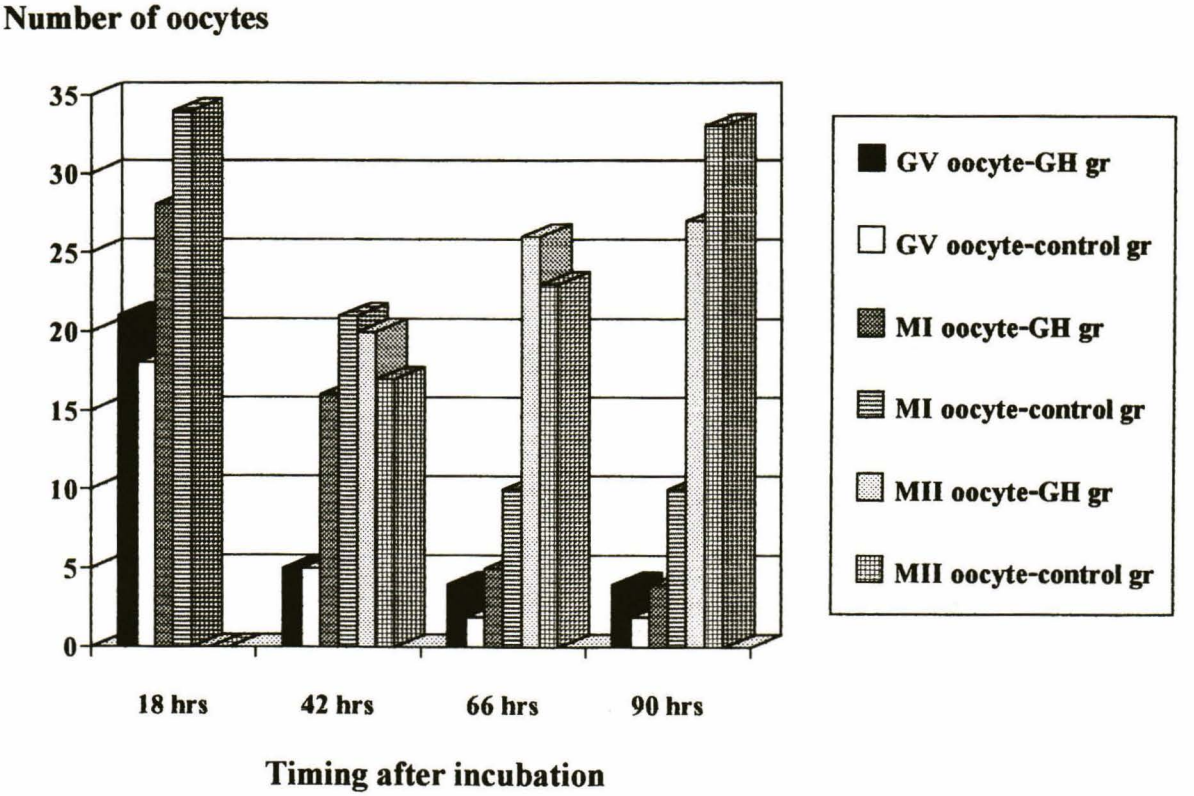

Fig. 4. Stage of oocytes after incubation.

Baseline characteristics of the owners' oocytes are shown in Table 1. The mean age of the patients in the growth hormone group was significantly higher than that of the control group (38.9 and 35.6 years). Other patients' characteristics, including phase of menstrual cycle, BMI and oocyte diameter were similar in both groups. 3 oocytes in growth hormone group and 2 oocytes in the control group were lost or damaged during incubation. The number of degenerated oocytes after 90 hours was 17 (32.7%) in the growth hormone group and 18 (33.9%) in the control group. After 90 hours of *in vitro* maturation, the number of oocytes which had germinal vesicle breakdown was 40 (76.9%) in the growth hormone group, 42 (79.2%) in the control group. The maturation rate of germinal vesicle stage oocyte was not significantly different between both groups.

DISCUSSION

Retrieval of immature human oocytes followed by *in vitro* maturation and fertilization has had limited study with poor outcome[7,11-13]. The majority of *in vitro*-matured unstimulated oocyte studies were performed in animals.[14-18] It is worthwile to carry on further investigation since *in vitro* maturation offers a number of advantages, such as prevention of hyperstimulation syndrome, reduced treatment costs and relief from anxiety over the potential long-term side effects of ovarian stimulation drugs.

One of the most important factors regulating the quality of oocytes maturity *in vitro* was the culture system used for *in vitro* maturation. The most commonly used design is a culture system supplemented with hormones and serum which is accepted as the optimal culture media for *in vitro* maturation[11,13].

Growth hormone is a single chain, 191 amino acid protein, and its regulatory effects on growth and metabolism have been recognized for many years [19,20]. The possible involvement of growth hormone in the regulation of ovarian maturation and development in mammals has been suggested by several investigators[21-23]. The stimulatory effect of growth

Table 1. Baseline characteristics of GV stage oocytes

	Growth hormone gr	Control gr	P-value
Age of patient (yrs)	38.9 ± 5.9	35.6 ± 8.8	0.02*
Day of menstrual cycle			
Proliferative	27	32	
Luteal	27	22	0.33**
BMI (kg/m^2)	24.6 ± 3.9	23.4 ± 3.3	0.09*
Indication for surgery			
Myoma	35	28	
Ovarian tumor	9	16	
Others	10	10	
Oocytes diameter	147.5 ± 20.4	147.5 ± 11.1	0.99*

* student *t*-test, ** Chi square test

Table 2. Outcome of oocytes maturation

	Growth hormone group (52 oocytes)	%	Control group (53 oocytes)	%
Maturation rate (metaphase II oocytes)	27	51.90	25*	47.20
Germinal vesicle breakdown rate (metaphase I oocytes)	40	76.90	42**	79.20

P-value 0.626 (Chi square test)*
P-value 0.774 (Chi square test)**

hormone on *in vitro* maturation of rat[24], rabbit[25], pig[26] oocytes has been reported. Growth hormone mediates its effect *via* growth hormone receptor. The mechanism by which growth hormone exerts its effect on *in vitro* maturation of mammalian oocytes has been found to be different among species. In bovine oocytes, the stimulatory effect of growth hormone is exerted through the cumulus cells and not mediated by insulin-like growth factor I (IGF I). The cAMP signal transduction pathway is involved in stimulatory effect of growth hormone on oocyte maturation. Several lines of evidence indicate that a certain threshold level of cAMP within the oocyte maintains meiotic arrest[2,27]. With regard to the resumption meiosis, several hypotheses have been formulated. A temporary increase in the cAMP concentration in cumulus cells due to hormone receptor-binding cells may 1) induce down-regulation of gap junctions between cumulus cells and the oocyte, thus, blocking the communication between them, 2) generate a positive GVBD-inducing signal in the cumulus cells that is transmitted to the oocyte through the gap junctions, or 3) result in transport of cAMP from the cumulus cells to the oocyte, causing a transiently enhanced cAMP concentration in the oocyte that triggers the resumption of meiosis[27]. In rabbit oocytes, growth hormone may stimulate follicular growth *via* growth hormone and lactogenic receptor as well. The stimulatory effect of growth hormone on *in vitro* maturation are thought to result from increasing concentration of IGF I[25]. However, in rat oocytes, there is evidence that the growth hormone effect is mediated by IGF I but not mediated by lactogenic receptor and enhances the effect of gonadotropin[24].

The stimulatory effect of growth hormone exerted on human *in vitro* maturation oocytes is still unknown. Although there were no significant differences in the proportion of oocytes that reached metaphase II with the presence or absence of growth hormone, it was noteworthy that the higher rate of maturation was observed in oocytes cultured in growth hormone. The result of IVM oocytes was different from studies in bovine and rat oocytes[21,22]. There are several reasons that may explain this results. The first is the status of the growth hormone receptor in the human oocytes. There has been, up to now, no evidence to show either presence or absence growth hormone receptor in human oocytes. The second reason, if human oocytes contain growth hormone

receptor, which condition is optimal for growth hormone to exert its stimulatory effect? What is the optimal dose of growth hormone in which it can work? In the present study, the authors chose 1,000 ng/ml growth hormone which show the optimal dose response in bovine oocyte study[27]. Unfortunately, the human oocytes response is not similar to bovine oocytes response. From the study of rabbit oocytes, the stimulatory effect of growth hormone was dose dependent[25]. Then, 1,000 ng/ml growth hormone may not be the appropiate dose for human oocytes maturation. It may need further study to seek the optimal dose.

Data from the present study shows different mean age of patients between the growth hormone group and the control group. It is clear that increased maternal age has detrimental effects on oocytes quality. In contrary to oocyte quality, Whitacre KS et al reported no statistical difference in oocytes maturation from patients >40 or <40 years of age[28].

An additional study is warranted to further investigate the effects of these and other variables on the maturation of human oocytes *in vitro*. Future studies to identify factors, which may enhance the oocyte's ability to mature *in vitro*, are being pursued. Understanding the myriad of variables associated with the collection and maturation of immature oocytes is essential if these applications are to reach their full potential.

ACKNOWLEDGEMENT

The study was supported by the Ratchadapiseksompotch fund, Faculty of Medicine, Chulalongkorn University.

(Received for publication on February 28, 2002)

REFERENCES

1. Baker TG. Quantitative and cytological study of germ cells in human ovaries. Proc R Soc 1963; 158: 417-33.
2. Cha KY, Chian RC. Maturation *in vitro* immature human oocytes for clinical use. Hum Reprod Update 1998; 4: 103-20.
3. Whelan JG, Vlahos NF. The ovarian hyperstimulation syndrome. Fertil Steril 2000; 73: 883-96.
4. Canipari R. Oocyte-granulosa cell interactions. Human Reprod Update 2000; 6: 279-89.
5. Gomez E, Tarin JJ, Pellicer A. Oocyte maturation in humans: The role of gonadotropins and growth factors. Fertil Steril 1993; 60: 40-6.
6. Das K, Stout LE, Hensleigh HC, Tagatz GE, Phipps WR, Leung BS. Directive effect of epidermal growth factor on the cytoplasmic maturation of mouse and human oocytes. Fertil Steril 1991; 55: 1000-4.
7. Dandekar PV, Martin MC, Glass RH. Maturation of immature oocytes by coculture with granulosa cells. Fertil Steril 1991; 55: 95-9.
8. Tonetta SA, di Zerega GS. Intragonadol regulation of follicular maturation. Endocr Res 1989; 10: 205-29.
9. Apa R, Lanzone A, Miceli F. Growth hormone releasing factor stimulates meiotic maturation in follicle- and cumulus-enclosed rat oocytes. Mol Cell Endocrinol 1995; 112: 195-201.
10. Izadyar F, Colenbrander B, Bevers MM. *In vitro* maturation of bovine oocytes in the presence of growth hormone accelerates nuclear maturation and promotes subsequent embryonic development. Mol Reprod Dev 1996; 45: 372-7.
11. Mikkelsen AL, Smith S, Lindenberg S. Impact of oestradiol and inhibin A concentrations on pregnancy rate *in vitro* oocyte maturation. Hum Reprod 2000; 15: 1685-90.
12. Thornton MH, Francis MM, Paulson RJ. Immature oocyte retrieval : Lessons from unstimulated IVF cycled. Fertil Steril 1998; 70: 647-50.
13. Alak BM, Coskun S, Friedman CI, Kennard EA, Kim HM, Seifer DB. Activin A stimulates meiotic maturation of human oocytes and modulates granulosa cell steriodogenesis in culture. Fertil Steril 1998; 70: 1126-30.
14. Sirard MA, Bilodeau S. Effects of granulosa cell co-culture on *in vitro* meiotic resumption of bovine oocytes. J Reprod Fertil 1990; 89: 459-65.
15. Kotsuji F, Kubo M, Tominaga T. Effects of interactions between granulosa and thecal cells on meiotic arrest in bovine oocytes. J Reprod Fertil 1994; 100: 151-6.
16. Motlik J, Sutovsky P, Kalous J, Kubelka M, Moos J, Schultz RM. Co-culture with pig membrana granulosa cells modulates the activity of cdc2 and MAP kinase in maturing cattle oocytes. Zygote 1996; 4: 247-56.
17. Fukui Y, Ono H. Effects of sera, hormones and granulosa cells added to culture medium for *in vitro* maturation, fertilization, cleavage and development of bovine oocytes. J Reprod Fertil 1989; 86: 501-6.
18. Schramm RD, Bavister BD. Granulosa cells from follicle stimulatinghormine-primed monkeys enhance the developmental competence of *in vitro*-matured oocytes from non-stimulated rhesus monkeys. Hum Reprod 1996; 11: 1698-702.
19. Cheek DB, Hill DE. Effect of growth hormone on cell and somatic growth. In: Greep RO, Astwood EB, editors. Handbook of physiology, endocrinology, the pituitary gland and its neuro endocrine control. Washington DC: AM Physiol Soc, 1974: 159-85.
20. Gluckman PD, Breier BH, Davis SR. Physiology of the somatotropic axis with particular reference to the ruminant. J Dairy Sci 1987; 70: 442-66.
21. Jia XC, Kalmijin J, Hsueh AJW. Growth hormone enhances follicle-stimulating hormone-induced differentiation of cultured rat granulosa cells. Endocrinology 1986; 118: 1401-9.
22. Hutchinson LA, Findlay JK, Herington AC. Growth hormone and insulin-like growth factor-I accelerate PMSG-induced differentiation of granulosa cells. Mol Cell Endocrinol 1988; 55: 61-9.
23. Manson HD, Martkanien H, Beard RW, Fulghesu AM, Caruso A, Mancuso S. Direct gonadotropin effect of growth hormone on oestradiol production by human granulosa cells. J Endocrinol 1990; 126: R1-4.
24. Apa R, Lanzone A, Miceli F, et al. Growth hormone induces *in vitro* maturation of follicle-and cumulus-enclosed rat. Mol Cell Endocrinol 1994; 106: 207-12.
25. Yoshimura Y, Iwashita M, Karube M, et al. Growth hormone stimulates follicular development by stimulating ovarian production of insulin-like growth factor-I. Endocrinology 1994; 135: 887-94.
26. Hagen DR, Graboski RA. Effects of porcine pituitary growth hormone (pGH) on cytoplasmic maturation of procine oocytes *in vitro*. J Anim Sci 1990; 68: 446.
27. Izadyar F, Colenbrander B, Bevers MM. Stimulatory effect of growth hormone on *in vitro* maturation of bovine oocytes is exerted through the cyclic adenosine 3', 5'-monophosphate signaling pathway. Biol Reprod 1997; 57: 1484-9.
28. Whitacre KS, Seifer DB, Friedman CI, et al. Effects of ovarian source, patient age, and menstrual cycle phase on *in vitro* maturation of immature human oocytes. Fertil Steril 1998; 70: 1015-21.

ผลการใช้โกร๊ธ ฮอร์โมนเพาะเลี้ยงเซลล์ไข่มนุษย์ที่ไม่ได้รับการกระตุ้นด้วยฮอร์โมน

อารีย์พรรณ โสภณสฤษฏ์สุข, พ.บ., สมชาย สุวจนกรณ์, วท.บ., พ.บ.*, กำธร พฤกษานานนท์, พ.บ.*, วิสันต์ เสรีภาพงศ์, พ.บ.*, วิชชุดา อานนท์กิจพานิช, วท.ม.*, เดือนทิพย์ ชมภูรัตน์, วท.บ.*, ปราณี นำชัยศรีค้า, วท.บ.*, วิสุทธิ์ บุญเกษมสันติ, พ.บ.*, ประมวล วีรุตมเสน, พ.บ.**

วัตถุประสงค์ : เพื่อศึกษาผลของโกร๊ธ ฮอร์โมนต่อการเจริญของเซลล์ไข่มนุษย์ที่เก็บจากรังไข่ที่ไม่ได้รับการกระตุ้นด้วยฮอร์โมน

รูปแบบการศึกษา : randomized controlled study

วัสดุและวิธีการ : ทำการเก็บไข่ระยะ germinal vesicle จากรังไข่ผู้ป่วยจำนวน 47 คน ที่เข้าทำการผ่าตัดทางนรีเวช โดยเจาะดูดเซลล์ไข่จากรังไข่ขณะที่ทำการผ่าตัดหรือเจาะดูดเซลล์ไข่จากรังไข่ที่ตัดออกมาแล้วเพาะเลี้ยงเซลล์ไข่ใน Tissue culture medium 199 ที่เสริมด้วยโซเดียม ไพรูเวท ฮอร์โมน FSH LH ยาปฏิชีวนะและเซรุ่มสังเคราะห์ แบ่งสุ่มเซลล์ไข่เป็น 2 กลุ่ม คือกลุ่มควบคุม เพาะเลี้ยงเซลล์ไข่ในสารเพาะเลี้ยงดังกล่าวข้างต้น และกลุ่มทดลองคือใส่ growth hormone ขนาด 1,000 นาโนกรัมต่อลูกบาศก์เซนติเมตร เพาะเลี้ยงเซลล์ไข่ในตู้อบที่อุณหภูมิ 37 องศาเซลเซียส ในอากาศที่มีกาซคาร์บอนไดออกไซด์ผสมอยู่ร้อยละ 5 ประเมินนิวเคลียสเมื่อเวลา 18, 42, 66, 90 ชั่วโมง หลังการเพาะเลี้ยง

ตัววัดหลัก : อัตราการเกิด metaphase II และ germinal vesicle breakdown

ผลการศึกษา : ไม่มีความแตกต่างของอัตราการเกิด metaphase II และ germinal vesicle breakdown ระหว่างกลุ่มทดลองและกลุ่มควบคุม โดยพบว่ากลุ่มทดลองมีเซลล์ไข่จากระยะ germinal vesicle ที่สามารถเจริญถึงระยะ metaphase II จำนวน 27 จาก 52 ใบ (ร้อยละ 51.9) และในกลุ่มควบคุมมีเซลล์ไข่จากระยะ germinal vesicle ที่สามารถเจริญถึงระยะ metaphase II จำนวน 25 จาก 53 ใบ (ร้อยละ 47.2) และอัตราการเกิด germinal vesicle breakdown ในกลุ่มทดลองและกลุ่มควบคุมเท่ากับ 76.9 และ 79.2 ตามลำดับ

สรุปผลการศึกษา : อัตราการเจริญของเซลล์ไข่มนุษย์ในห้องทดลองเมื่อเลี้ยงในสารเพาะเลี้ยงที่มีหรือไม่มีโกร๊ธ ฮอร์โมนไม่แตกต่างกัน

คำสำคัญ : ฮอร์โมนเร่งการเจริญ, การเจริญของเซลล์ไข่, เซลล์ไข่ในระยะเริ่มต้น

อารีย์พรรณ โสภณสฤษฏ์สุข, สมชาย สุวจนกรณ์, กำธร พฤกษานานนท์, และคณะ จดหมายเหตุทางแพทย์ ฯ 2545; 85: 907-914

*ภาควิชาสูติศาสตร์-นรีเวชวิทยา, คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพ ฯ 10330