Short Communication

SUPEROVULATION RESPONSES IN RABBITS TO DIFFERENT DOSES OF FOLLICLE STIMULATING HORMONE

Mongkol Techakumphu[1*] Pranee Numchaisrika[2] Vaneda Suktrakun[1] Suthai Phanitkitcharoen[1] Astasit Kaewnanuer[1] Somchai suwajanakorn[2]

Abstract

Mongkol Techakumphu[1*] Pranee Numchaisrika[2] Vaneda Suktrakun[1] Suthai Phanitkitcharoen[1] Astasit Kaewnanuer[1] Somchai suwajanakorn[2]

SUPEROVULATION RESPONSES IN RABBITS TO DIFFERENT DOSES OF FOLLICLE STIMULATING HORMONE

The objective of the experiment was to study the number of oocytes recovered, their quality and the recovery rate after superovulation, in order to use the oocytes, as a cytoplasm recipient in a nuclear transfer program. A total of 38, New Zealand White mature, doe rabbits were divided into 3 groups, according to the chosen dose of Follicle Stimulating Hormone (FSH); 21 mg (group A), 28 mg (group B) and 40 mg (group C). Five IM injections of FSH were given at 12 hr interval by increasing doses for group A and B and a constant dose for group C. The ovulation was induced by giving 100 iu human chrorionic gonadotropin (hCG) and mating by a vasectomized buck. The oocytes were collected following ovariectomy and direct flushing. The number of corpora lutea (CL), non hemorrhagic and hemorrhagic follicles were counted. The number of CL were 24.5±8.8, 21.3±11.6 and 20.4±8.6 ($P>0.05$) respectively. The recovery rate in group A, (77.7%) was higher than those of group B, (55.7%) and C, (50.9%) ($P<0.001$). A higher number of recovered oocytes was found in group A, (19.1±8.0) than those of group B, (11.8±7.0) ($P<0.01$) and group C, (10.4±8.3) ($P<0.001$) respectively. More than 90% of recovered oocytes in every group were morphologically normal. This study showed that the dose of FSH influenced the oocyte recovery rate and the number of oocyte per female.

Keywords : oocyte, superovulation, FSH, rabbit

[1]**Department of Obstetrics Gynaecology and Reproduction, Faculty of Veterinary Sciences, Chulalongkorn University, Bangkok 10330.**

[2]**Reproductive Medicine Unit, Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330.**

[1]ภาควิชาสูติศาสตร์ เธนุเวชวิทยาและวิทยาการสืบพันธุ์สัตว์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพฯ 10330

[2]หน่วยชีววิทยาการเจริญพันธุ์ ภาควิชาสูติศาสตร์ นรีเวชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพฯ 10330

*ผู้รับผิดชอบบทความ

บทคัดย่อ

มงคล เตชะกำพุ[1] ปราณี นำชัยศรีค้า[2] วนิดา สุขตระกูล[1] สุทัย พาณิชย์กิจเจริญ[1] อรรถสิทธิ์ แก้วนาเหนือ[1] สมชาย สุวจนกรณ์[2]

ขนาดของฟอลลิเคิล สติมูเลติง ฮอร์โมนต่อการตอบสนองในการกระตุ้นเพิ่มการตกไข่ในกระต่าย

จุดประสงค์ของงานวิจัยนี้เพื่อศึกษาจำนวนการตกไข่และจำนวนโอโอไซต์ที่ได้หลังกระตุ้นเพิ่มการตกไข่ด้วยฟอลลิเคิล สติมูเลติง ฮอร์โมน เพื่อใช้ในการย้ายฝากนิวเคลียสด้วยเซลล์โซมาติก โดยทดลองในกระต่ายเพศเมียพันธุ์นิวซีแลนด์ไวท์ จำนวน 38 ตัว แบ่งเป็น 3 กลุ่มตามขนาดของฮอร์โมนที่ใช้ คือ กลุ่ม A ขนาด 21 มก. (n=15) , กลุ่ม B ขนาด 28 มก. (n=11) และกลุ่ม C ขนาด 40 มก. (n=12) แบ่งฉีดวันละ 2 ครั้ง (เช้า/เย็น) จำนวนทั้งหมด 5 ครั้ง และกระตุ้นให้มีการตกไข่โดยผสมด้วยพ่อพันธุ์ตัดต่อน้ำเชื้อ ร่วมกับฮิวแมน โคริโอนิก โกนาโดโทรปิน ขนาด 100 ไอยู เข้าหลอดเลือดดำ ทำการเก็บโอโอไซต์หลังผ่าตัดและชะล้างท่อนำไข่หลังผสม 16 ชั่วโมง ทำการเปรียบเทียบจำนวนการตกไข่ อัตราการเก็บโอโอไซต์และคุณภาพของโอโอไซต์ในแต่ละกลุ่ม ผลการศึกษาพบว่าจำนวนการตกไข่ในแต่ละกลุ่มไม่แตกต่างกัน เท่ากับ 24.5±8.6, 21.3±11.6 และ 20.4±8.6 ตามลำดับ (P>0.05) ส่วนอัตราการเก็บโอโอไซต์ในกลุ่ม A เท่ากับ 77.7% สูงกว่ากลุ่ม B และ C เท่ากับ 55.7% และ 50.9% ตามลำดับ (P<0.001) จำนวนโอโอไซต์ในกลุ่ม A ได้จำนวนสูงสุดเท่ากับ 19.1±8.0 แตกต่างกับกลุ่ม B เท่ากับ 11.8±7.0 (P<0.01) และกลุ่ม C เท่ากับ 10.4±8.3 โอโอไซต์ (P<0.01) ตามลำดับ โอโอไซต์มากกว่า 90% มีลักษณะปกติและเป็นชนิดที่พร้อมปฏิสนธิโดยไม่แตกต่างกันในแต่ละกลุ่ม จากการศึกษานี้แสดงให้เห็นว่าขนาดของฟอลลิเคิล สติมูเลติง ฮอร์โมนไม่มีผลที่แตกต่างกันในแง่ของจำนวนการตกไข่และคุณภาพของโอโอไซต์ที่เก็บได้ แต่อัตราการเก็บโอโอไซต์และจำนวนโอโอไซต์แตกต่างกัน

คำสำคัญ : โอโอไซต์ การกระตุ้นเพิ่มการตกไข่ ฮอร์โมน เอฟเอสเอช กระต่าย

Introduction

In a nuclear transfer program, oocytes at the metaphase II stage were used as a cytoplasm recipient which was capable of reprograming the nuclear donor and giving the highest success rate (Willadsen, 1986). Rabbits are one of the better animals for nuclear transfer models as they provide a high number of recipient oocytes per donor. Rabbit oocytes can be produced though the technique of superovulation using an exogenous gonadotropin hormone and collecting the ovum by direct tubal flushing (Kauffman et al., 1998; Yang, 1991). Two types of gonadotropins; Pregnant Mare Serum Gonadotropin (PMSG) and Follicle Stimulating Hormone (FSH) can be used to superovulate. The treatment with PMSG can often

induce an unsatisfactory result while Techakumphu (1986) found that Follicle Stimulating Hormone (FSH) was effective in providing a high number of embryos per animal. FSH should be given twice a day at a 12 hr interval, for 3 days and Luteinizing Hormone (LH) on the following day, at the same time as natural or artificial mating for successful ovulation induction (Tsunoda et al., 1981). The objective of this experiment was to study the oocyte recovery rate and its quality after superovulation in order to use these oocytes as cytoplasm recipients in a nuclear transfer program.

Materials and Method

Rabbit:

A total of 38, six-month-old, New Zealand White, rabbit does, weighing ≥3.0 kg were used in the experiment. These rabbits were bought from the colony kept at the Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University and were housed in individual cages at 25°C with 12hrs of artificial light daily. An ad libitum, 14% protein, concentrated feed diet and fresh water was given. Two vasectomized bucks were prepared for mating. The experiment was conducted between October 2000 and December 2001.

Superovulation program:

The animals were injected with Follicle Stimulating Hormone (FSH, Vetapharm(r), Australia) intramuscularly with five injections at 12 hr intervals starting in the evening and continuing for 3 days. Three experimental groups were tested as followed: Group A: 21 mg FSH (n=15) (3,3+3,6+6), Group B: 28 mg FSH (n=12) (4,4+4,8+8) and Group C: 40 mg FSH (n =11) (8, 8+8, 8+8) (Fig.1). Ovulations were induced by mating with a vasectomized buck, together with 100 iu of intravenous Human Chorionic Gonadotropin (hCG, Chorulon(r), Intervet, The Netherlands) (Schmidt et al., 1992). The dose of FSH differed from that used previously (Techakumphu, 1986). The rabbit was laparotomized under general anesthesia, 16hrs post coitus and its genital tract, placed in 20 ml Phosphate Buffer Saline (PBS), was immediately brought to the animal experimental laboratory of the Reproductive Medicine Unit, Faculty of Medicine, for oocyte flushing.

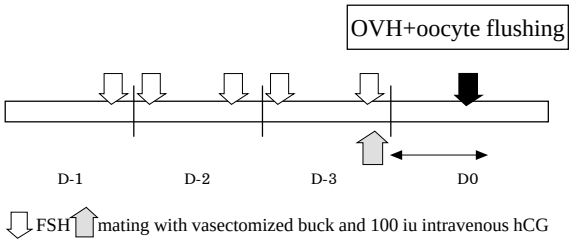

Figure 1. A schematic of the timelines of FSH-HCG treatment in the rabbits

Oocyte collection:

The genital tract was washed once in fresh PBS and each side of oviduct and 2 cm of uterine horn were dissected from the surrounding tissue. The oocytes were collected by using direct flushing of the oviduct as described previously by Techakumphu (1986). A blunt-ended 18g needle was inserted through the uterine end to the fimbria and flushed with 10 ml of Phosphate Buffer Saline (PBS)+2% Fetal Calf Serum (FCS). The flushing was performed twice and the media

were recovered in a 35-mm petri dish. The oocytes were searched for immediately, under a 10X stereomicroscope and those recovered were placed in TCM 199 Hepes+10% FCS. They were then washed twice in fresh media and incubated in 0.5% Hyarulonidase (Sigma, USA) for 30 min. After incubation, the cumulus cells around the oocyte were removed by pipetting. The oocytes were examined under a microscope to investigate the 1st polar body which classified them as matured oocytes. The number of corpora lutea, non hemorrhagic and hemorrhagic follicles was counted. The oocyte recovery rate was compared with the number of corpora lutea in both ovaries.

Statistical analysis:

The number of ovulation, follicles and recovered oocytes per donor were compared between the programs using one-way analysis of variance and the recovery rates by the Chi-square test.

Result

Table 1 shows that the mean number of ovulations and the mean number of non hemorrhagic and hemorrhagic follicles per donor in each program differed significantly (P>0.05), while the number of recovered oocytes per donor were highest in group A rather than B or C (P<0.01). A high variation in ovulation rates and recovered oocytes were observed in all treatments. The recovery rate was also highest in group A compared to groups B and C (P<0.001). More than 90% of oocytes in all treated groups were morphologically normal with homogenous cytoplasm and a first polar body.

Table 1. Number of ovulations, follicles and recovered oocytes per female and % of recovery after superovulation (mean±SD) of groups A, B and C

Program	No. rabbit does	No. ovulations	No. follicles	No. recovered oocytes	% Recovery
A	15	24.5±8.6 (10-46)	8.9±7.4 (0-30)	19.1±8.0[a] (7-33)	77.7[d]
B	12	21.3±11.6 (4-31)	7.5±5.6 (0-16)	11.8±7.0[b] (2-23)	55.7[e]
C	11	20.4±8.6 (14-39)	10.5±7.7 (1-25)	10.4+8.3[c] (4-23)	50.9[e]

[a, b] Significant difference, $P<0.05$ (chi^2 test)

[a, c] Significant difference, $P<0.01$ (chi^2 test)

[b, c] no significant difference, $P>0.05$ (chi^2 test)

[d, e]; Significant difference, $P<0.001$ (analysis of variance)

(range)

Discussion

In this experiment, it was shown that the treatment of 21, 28 or 40 mg FSH with 100 IU hCG provided an average response of 20 ovulations per female. The observation was comparable to Kauffman et al. (1998) who showed the mean (±SD) number of ovulations per female was 25.6±15.6 for the 3 d regimen and 23.5±8.4 for 4 d but it was lower than in our previous observation (Techakumphu, 1986). The difference came from various factors such as the environment, the management system, the type of gonadotropin, the number of injections, the methods of ovulation induction, the mating system, the age of donor etc. (Kenelly and Foote, 1965; Techakumphu, 1986; Techakumphu et al., 1987; Schmidt et al., 1992; Kauffman et al., 1998). It was remarked that a higher concentration of FSH in program B and C did not improve the number of ovulations but resulted in an increased number of non hemorrhagic and hemorrhagic follicles and a lower oocyte recovery rate. Our preliminary study which increased the dose of FSH to 48 mg in four rabbit does gave a significantly lower number of oocytes per female which the recovery rate of only 37.7%. Overstimulation tended to reduce the rate of oocyte recovery which was attributed partially to the effect of endogenous estrogen production on the rate of oocyte transport and partially to oocytes being trapped in the follicles (Kenelly and Foote, 1965). The failure of ova to leave the ruptured follicles was due to trapping in the follicular lumen or embedding in the granulosa cell of a ruptured follicle. Furthermore, the oocyte loss may also come from an excessively rapid passage through the oviduct induced by the FSH overstimulation as also mentioned by Greenwald (1961). This may explain the low recovery rates in rabbit receiving a high dose of FSH. A high variation of ovulation was also evident in every group either a low or a high dose of FSH. However, most recovered oocytes were mature as judged by the presence of the first polar body on the surface of oocyte and were morphologically suitable for using as recipient oocyte in a somatic nuclear transfer program (Techakumphu et al., 2001). In conclusion, the oocyte recipient can be produced from rabbits giving a moderate response from a low dose of FSH.

Acknowledgement

The Reproductive Medicine Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, supported this study. Our thanks to Dr. Sudson Sirivaidyapong and the staff of Obstetrics Clinic, Faculty of Veterinary Science for surgical support and Dr. Padet Tummaruk for statistical analysis.

References

Greenwald, G.S. 1961. A study of the transport of ova through the rabbit oviduct. Fertil. Steril. 12:80.

Kauffman, R.D., Schmidt, P.M., Rall, W.F. and Hoeg, J.M. 1998. Superovulation of rabbits with FSH alters *in vivo* development of vitrified morulae. Theriogenology. 50: 1081-1092.

Kenelly, and Foote, R.H. 1965. Superovulatory response of pre- and post-pubertal rabbits to commercially available gonadotropins. J. Reprod. Ferti. 9: 177-188.

Schmidt, P.M., Hollifiedl, V.M., Lin, X. and Wildt, D.E. 1992. Induced ovulation and adequate embryo recoveries in New Zealand White rabbits treated with a low PMSG/HCG dose or single, daily injections of FSH-P. Theriogenology. 37(1): 293. (Abstr.)

Techakumphu, M. 1986. Superovulation in rabbit. Thai J. Vet. Med. 16(4): 272-285.

Techakumphu, M. Wintenberger,-Torres, S. and Sevellec, C. 1987. Survival of rabbit embryos after synchronous and asynchronous transfer. Anim. Reprod. Sci. 12: 297-304.

Techakumphu M. , Numchaisrika, P., Ahnonkitpanit, V., Suwajanakorn, S., Pruksananonda, Boonkasemsanti, W. and Virutamasen, P. 2001. Preliminary study on somatic cell nuclear transfer in rabbit in Thailand. The 16th Annual Conference of The Royal Thai College of Obstrecian and Gynaecologist, 16-19 October 2001, 187.

Tsunoda, Y., Soma, T. and Sugie, T. 1981. Frozen storage of rabbit and hamster embryos. In: Frozen Storage of Laboratory Animals. G.H. Zeilmaker (ed.), Gustav Fischer Verlag. Stuttgart: 129-138.

Willadsen, S.M. 1986. Nuclear transplantation in sheep embryos. Nature. 320: 63-65.

Yang, X. 1991. Embryo cloning by nuclear transfer in cattle and rabbits. Embryo Transfer Newsletter. 9(4): 10-22.