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FULL PAPER

Follicular development after ovum pick-up and fertilizability of retrieved oocytes in postpartum dairy cattle

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Abstract

This study aimed to evaluate gonadotropin secretion and the developmental competence of follicular oocytes in dairy cattle during the early postpartum (PP) period. The number of follicles developed after transvaginal ultrasound-guided ovum pick-up (OPU) and fertilizability of retrieved oocytes were compared between cows in which the first dominant follicle (DF) ovulated (ovulated group, n=4) and did not ovulate (non-ovulated group, n=3), and between early PP (early PP group, n=2) and after the resumption of the estrous cycle (cyclic group, n=2). Follicular ablation was performed 2-4 days after the detection of DF in the second follicular wave PP. OPU was repeated 3-5 times at 3 or 4-day intervals from 3-4 days after the follicular ablation. At OPU, the follicles were enumerated and all those ≧5 mm in diameter were aspirated. Recovered oocytes were subjected to *in vitro* maturation and fertilization. Both criteria were similar between ovulated and non-ovulated groups, and between early PP and cyclic groups. These results suggest that FSH/LH secretions required for follicle recruitment and subsequent follicular growth during the early PP period are similar to those after resumption of the estrous cycle. They also indicate that follicular oocytes during the early PP period have developmental competence.

Key words : cattle, follicle, oocyte, ovum pick-up, postpartum.

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Introduction

Transvaginal ultrasound-guided ovum pick-up (OPU) in live cows allows repeated oocyte collection for the production of offspring using *in vitro* embryo production technology[14,19,21]. In commercial embryo transfer laboratories, the oocytes are usually retrieved from clinically infertile, yet genetically valuable cows[19]. Successful OPUs in pregnant cows[17] and prepubertal calves[7] have also been reported. However, there have been few reports on the collection of bovine embryos by OPU during the early postpartum (PP) period[22]. Thus, it is not known whether follicular oocytes in early PP have a developmental competence equivalent to that of oocytes from cyclic cows.

The ablation of existing ovarian follicles induces the secretion of follicle-stimulating hormone (FSH)[27] from the pituitary and recruitment of small follicles[11]. The growth and function of recruited follicles ≧4 mm in diameter largely depend on both FSH and luteinizing hormone (LH)[15], though recent studies have demonstrated important roles for local factors including insulin-like growth factor-I, the transforming growth factor β family and fibroblast growth factor[2,9,30,31]. Thus, the number and size of follicles and fertilizability of oocytes from follicles recruited after the aspiration of follicles might be affected by secretion of FSH and LH from the pituitary.

FSH secretion and follicular waves are undetectable 2-3 weeks before parturition[12]. Most dairy cows appear to develop their first dominant follicles (DFs) in the second week PP and they can be classified into three groups based on the fate of the first DF; ovulation, atresia and follicular cyst[3,24]. The ovulation of DFs is mainly regulated through the secretion of LH[4,10]. However, LH secretion has not been examined based on the fate of the first DF. Further, there is no report on the fertilizability of oocytes retrieved from cows in which the fate of the first DF differs.

Most dairy cows are not able to meet the energy requirements for milk production early in the lactation period and, therefore, experience a negative energy balance[3,4,10]. Moreover, the energy balance gradually recovers with time PP[3,4,10]. It has been believed that the negative energy balance results in low secretions of FSH/LH[10] and in the production of oocytes of poor quality[20]. Although several researchers have performed OPU one or more times in individual cows during different PP periods and examined the number and size of follicles recruited after OPU and quality of retrieved oocytes, results are inconsistent[13,16,28,29].

The aim of this study was to evaluate gonadotropin secretion and the developmental competence of follicular oocytes in dairy cattle during the early PP period. The number of follicles developed after OPU and the fertilizability of retrieved oocytes were compared between cows in which the first DF ovulated and did not ovulate, and between the early PP and after resumption of the estrous cycle.

Materials and Methods

Animals

Seven Holstein cows (six primiparous and one multiparous) were kept tied to stalls during the experimental period. They were fed silage, hay and concentrates according to Japanese nutritional standards for dairy cattle[1], and milked two times a day.

Follicular ablation and transvaginal ultrasound-guided ovum pick-up (OPU)

Follicular ablation and OPU were performed as described previously[23]. Briefly, the apparatus used consisted of an ultrasound device (EUB-405; Hitachi, Tokyo, Japan), a 6.5-MHz fingertip probe (Model EUP-F-331; Hitachi) and a handmade probe-carrier (50-cm long). A single-lumen needle (18 gauge, 60-cm long; Fujihira Industries, Tokyo) was used as an aspiration needle. The flushing medium was Dulbecco's phosphate-buffered saline supplemented with 1% calf serum (Gibco BRL, Gland Island, NY, USA), 0.05 mg/ml of streptomycin sulfate (Meiji Seika, Tokyo), 100 units /ml of penicillin G potassium (Banyu Pharmaceutical Co., Tokyo) and 10 IU/ml of heparin sodium (Heparin Upjohn 1000; Pharmacia & Upjohn, Tokyo). All follicles ≧5 mm in diameter were aspirated under a constant vacuum pressure of 75 mmHg (K-MAR-5000; Cook, Queensland, Australia). During the puncture of follicles, the aspiration needle was twisted 4 or 5 times at an angle of 180 degrees around its longitudinal axis to scoop out the entire wall of the follicle.

After OPU, oocytes were collected from the recovered follicular contents as described previously[23]. Retrieved oocytes were examined under a stereomicroscope and scored according to the morphological appearance of surrounding cumulus cells and ooplasm as described previously[16]; score 4, compact multilayered cumulus with more than three layers and a homogenous ooplasm; score 3, compact cumulus of one to two layers with homogenous ooplasm; score 2, less compact cumulus with an irregular ooplasm containing dark clusters; score 1, oocytes without cumulus cells, with expanded cumulus cells or with a degenerated ooplasm, regardless of the presence of cumulus cells.

In vitro maturation (IVM) and fertilization (IVF) of oocytes

The oocytes given scores of 2-4 were subjected to IVM and IVF procedures[26] individually with some modifications. Briefly, each oocyte was cultured for 22 hr in a 10-μl drop of HEPES-buffered TCM 199 (Gibco Laboratories, Grand Island, NY, USA) supplemented with 10% fetal calf serum (Gibco), 0.02 units /ml of FSH (Sigma Co., St. Louis, MO, USA), 1 μg/ml of estradiol-17β (Sigma), 0.2 mM sodium pyruvate (Sigma) and 50 μg/ml of gentamicin sulfate (Sigma). IVF was performed using frozen semen from a single ejaculate of a Holstein bull. After thawing, motile sperm were separated using a Percoll gradient (45 and 90%). Cumulus-oocyte-complexes were co-incubated for 18 hr with sperm (5×10^6 cells/ml) in a 20-μl drop of modified Brackett and Oliphant's medium supplemented with 3 mg/ml of fatty acid-free BSA (Sigma), 2.5 mM theophylline (Sigma) and 10 μg/ml of heparin (Sigma). Cultures for IVM and IVF were maintained at 39℃ in humidified air with 5% CO_2. After the removal of cumulus cells, inseminated oocytes were fixed with an acetic acid and ethanol mixture (1:3) overnight, and stained with 1% aceto-orcein. Sperm penetration was examined under a phase-contrast microscope. Oocytes were considered to be penetrated when they had an enlarged sperm head or male pronucleus with corresponding sperm tail. Normal fertilization was determined by the presence of a pair of pronuclei and a corresponding sperm tail.

Experimental design

In the first experiment, the ovaries of seven cows were examined daily using an ultrasound device (SSD620; Aloka, Tokyo) and a 5-MHz rectal linear-array probe (UST-588 U-5; Aloka) from 7 - 8 days after calving. Follicular development and oocyte fertilizability were compared between cows in which the first DF ovulated (ovulated group, n = 4) and did not ovulate (non-ovulated group, n = 3). A DF was defined as a follicle ≧10 mm in diameter and at least 2 mm larger than other follicles[25]. All follicles ≧ 5 mm in diameter were ablated 2 - 4 days after the detection of a DF in the second follicular wave PP. OPU was repeated 3 times at 3 or 4- day intervals (Monday and Thursday) from 3 or 4 days after the ablation.

In the second experiment, follicular development and oocyte fertilizability in early PP (early PP group) were compared with those after resumption of the estrous cycle (cyclic group). Two out of three cows of the non-ovulated group in the first experiment were subjected to follicular ablation 5 or 6 days after the onset of estrous. OPU was initiated from 4 days after the ablation, and was repeated 4 or 5 times at 3 or 4 - day intervals. Follicular development and oocyte fertilizability were compared to those during the early PP period.

Preceding each OPU, the number of small (5 - 9 mm in diameter) and large (≧10 mm in diameter) follicles was recorded throughout the study. The timing of follicular ablation and initiation of OPU in each group is given in Table 1.

Statistical analysis

The data on follicle numbers were subjected to an unpaired Student's t-test. Differences in the proportion of oocytes with each morphological score between the ovulated and non-ovulated groups, and between early PP and cyclic groups were analyzed with the chi-square test. The proportions of penetrated and normally fertilized oocytes in the ovulated and non-ovulated groups, and in the early PP and cyclic groups were analyzed using Fisher's exact test. Data analysis was performed using Stat View software (Abacus Conceptus Inc., Berkeley, CA, USA).

Results

The timing of the ablation of DFs in the second follicular wave PP and the initiation of OPU differed between the ovulated and non-ovulated groups (Table 1). As shown in Fig. 1, there were no differences in the mean numbers of the large, small and total follicles between the ovulated and non-ovulated groups (2.0 ± 1.2 vs. 1.6 ± 1.7, 6.3 ± 2.7 vs. 7.8 ± 2.8 and 8.3 ± 2.1 vs. 9.4 ± 2.3, mean ± SD, respectively) or between the early PP and cyclic groups (1.8 ± 1.2 vs. 1.1 ± 0.8, 5.7 ± 3.1 vs. 6.9

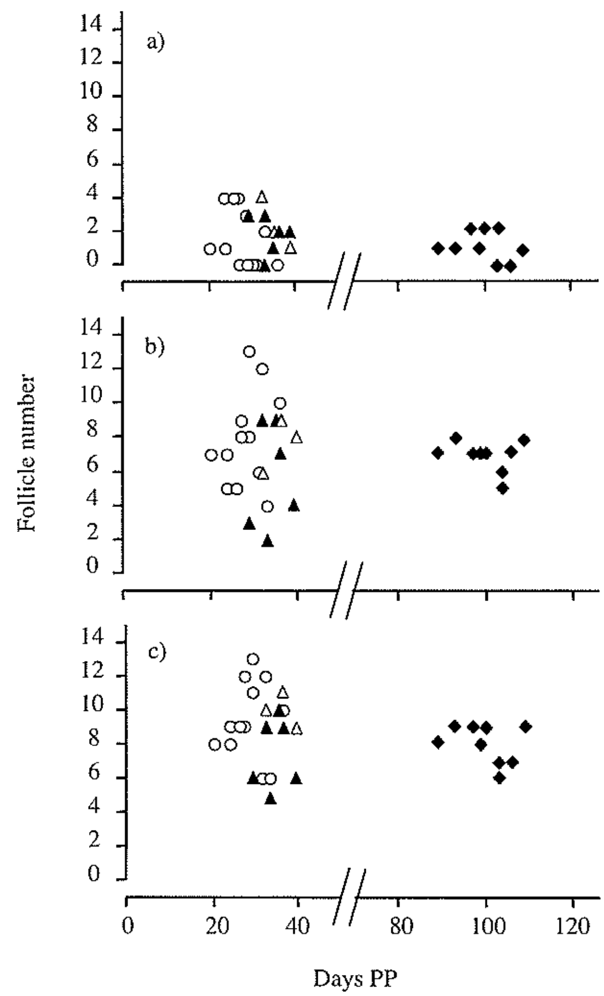

Fig. 1. The distribution of the number of follicles at OPU throughout the experimental period. Panels a), b) and c) give the number of large, small and total follicles, respectively. ○, △ and ◆ indicate the values for ovulated, non-ovulated and cyclic groups, respectively. ▲ indicates the values for two cows used as the early PP group.

Table 1. Timing of follicular ablation and initiation of OPU

Group [a]	No. of cows	Timing (days after calving)	
		Follicular ablation	Initiation of OPU [b]
Ovulated	4	17 to 25	20 to 29
Non-ovulated	3	26 to 29	29 to 32
Early PP	2 [c]	26 and 29	29 and 32
Cyclic	2 [c]	85 and 95	89 and 99

a) Cows in which the first DF ovulated (ovulated group) and did not ovulate (non-ovulated group). Cows in early postpartum (early PP group) and after resumption of the estrous cycle (cyclic group).
b) OPU was repeated three times in each cow of the ovulated and non-ovulated groups. In the cyclic group, OPU was performed four or five times.
c) Two out of three cows in the non-ovulated group.

± 0.9, 7.5 ± 2.1 vs. 8.0 ± 1.1, respectively). The variations in the number of follicles in each size category were greater in the ovulated and non-ovulated groups than in the cyclic group.

No differences in the proportion of oocytes with each morphological score or in penetration and normal fertilization rates were found between the ovulated and non-ovulated groups (Table 2, $p > 0.05$), and between the early PP and cyclic groups (Table 3, $p > 0.05$). The majority (55-60%) of retrieved oocytes received a score of 2, and the proportions of oocytes given scores of 2, 3 or 4, which were subjected to IVM-IVF, were 70-80%, regardless of the fate of the first DF and PP period (Tables 2 and 3). More than 80% of the inseminated oocytes were penetrated, and about 55% of inseminated oocytes had a pair of male and female pronuclei in all groups.

Table 2. Quality and fertilizability of the oocytes recovered by OPU: comparison between the ovulated and non-ovulated groups

	Group [a]	
	Ovulated	Non-ovulated
No. of oocytes recovered	31	31
% of oocytes with		
Score 4 [b]	16.1	19.4
3	9.7	6.5
2	54.8	58.1
1	19.4	29.0
No. of oocytes inseminated	25	20
% of penetrated oocytes with		
2 PN	56.0	65.0
ESH	24.0	30.0
Polyspermy	4.0	0
Total	84.0	95.0

a) See Table 1.
b) Score 4, compact multilayered cumulus with more than three layers and homogenous ooplasm; score 3, compact cumulus of one to two layers with homogenous ooplasm; score 2, less compact cumulus with an irregular ooplasm containing dark clusters; score 1, oocytes without cumulus cells, with expanded cumulus cells or with a degenerated ooplasm, regardless of the presence of cumulus cells.
c) 2 PN: a pair of pronuclei with a corresponding sperm tail.
ESH: an enlarged sperm head with a corresponding sperm tail.

Table 3. Quality and fertilizability of oocytes recovered by OPU: comparison between the early PP and cyclic groups

	Group [a]	
	Early PP	Cyclic
No. of oocytes recovered	19	26
% of oocytes with		
Score 4 [b]	10.5	7.7
3	0	11.5
2	63.2	61.5
1	26.3	19.2
No. of oocytes inseminated	13	21
% of penetrated oocytes with		
2 PN	61.5	57.1
ESH	30.8	38.1
Polyspermy	0	0
Total	92.3	95.2

a) See Table 1.
b, c) See footnote of Table 2.

Discussion

The present study revealed that fertilizable oocytes could be recovered from dairy cows by OPU during the early PP period. A previous study demonstrated that 18% of oocytes retrieved from dairy cattle before the resumption of the estrous cycle developed into blastocysts after *in vitro* production[22]. In beef cattle, the oocytes at the first ovulation had a normal fertilizability and developmental competence[6]. Although the developmental competence of recovered oocytes was not determined in the present study, it seems possible to produce calves using oocytes retrieved from cows during the early PP period with OPU techniques.

In cattle, follicular growth is dependent on both FSH and LH actions. In the present study, the number of small follicles (5-9 mm in diameter) in cows in which the first DF ovulated (ovulated group) was similar to that in cows in which the first DF did not ovulate (non-ovulated group). Therefore, it was suggested that there was no difference in the secretion of FSH required for follicle recruitment between the two groups. Serum concentrations of FSH rose soon after parturition (4 - 5 days PP)[3] and anterior pituitary FSH levels did not change with time PP[18].

A previous study[4] showed that LH pulse frequency was higher in the cows in which the first DF ovulated than in cows in which it did not. However, the present study suggested that LH pulse frequency in the non-ovulated group might be similar to that in the ovulated group since there was no difference in large follicle (≧10 mm in diameter) populations between the two groups. In the current study, OPUs in the non-ovulated group were performed after the fate of the first DF was ascertained, and the initiation of OPUs was later in the non-ovulated group than the ovulated group (29-32 vs. 20-29 days PP). During this delay, LH pulse frequency in the non-ovulated group might increase.

In this study, oocytes were recovered from follicles ≧5 mm in diameter. No differences in the proportions of penetrated and normally fertilized oocytes were found between the ovulated and non-ovulated groups. It was reported that oocytes within follicles (≧3 mm in diameter) had developmental competence, regardless of follicular diameter and atretic level (among non-, intermediate and slightly atretic follicles)[5]. These results indicate that the follicles recruited after OPU could support oocyte growth and the acquisition of normal fertilizability.

There were no differences in the number of follicles in each size category between cows in the early PP period (early PP group) and after resumption of the estrous cycle (cyclic group). Our results were consistent with a previous study in which the total number of follicles ≧6 mm in diameter did not differ between early and mid-lactation[29]. Therefore, it was suggested that the secretion of FSH and LH required for follicle recruitment and subsequent follicular growth in the early PP period was similar to that after the resumption of the estrous cycle.

It has been proposed that oocyte quality is lower 80-100 days PP than in the early PP period[8]. If preantral follicles, which have granulosa cells active in the synthesis of nucleic acids and protein, are exposed to a severe negative energy balance early in the PP period, the gene expression in granulosa cells may be affected. This could result in the formation of dysfunctional follicles, which produce oocytes of poor quality. As 80-100 days PP corresponds to the period when these preantral follicles develop into antral or Graafian follicles, it is considered that oocyte quality may be lower 80-100 days PP than in the early PP period[8]. However, a previous study showed no difference in the developmental competence of oocytes recovered by OPU from dairy cows before and after resumption of the estrous cycle[22]. The present study also indicated that the fertilizability of oocytes retrieved by OPU did not differ between the early PP and cyclic groups. The deficit of energy in the present experimental animals may not be severe because the decrease in body condition score from parturition to around 30 days PP was small (data not shown). Therefore, the results of this study do not conflict with the previous hypothesis[8].

In conclusion, the present study revealed that there were no differences in the follicular development and fertilizability of oocytes from follicles recruited after OPU between the cows in which the first DF ovulated and did not ovulate, and between early PP and after resumption of the estrous cycle. These results suggest that FSH/LH secretions required for follicle recruitment and subsequent follicular growth during the early PP period are similar to those after the resumption of the estrous cycle. They also indicate that follicular oocytes during the early PP period have developmental competence.

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References

1) Agriculture, Forestry and Fisheries Research Council Secretariat. 1999. *Japanese Feeding Standard for Dairy Cattle*. Japan Livestock Industry Association, Tokyo, Japan (in Japanese).

2) Bao, B. and Garverick, H. A. 1998. Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves : A review. *J. Anim. Sci.*, 76 : 1903-1921.

3) Beam, S. W. and Butler, W. R. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. *Biol. Reprod.*, 56 : 133-142.

4) Beam, S. W. and Butler, W. R. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *J. Reprod. Fertil.*, 54 (*Suppl.*) : 411-424.

5) Blondin, P. and Sirard, M. A. 1995. Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. *Mol. Reprod. Dev.*, 41 : 54-62.

6) Breuel, K. F., Lewis, P. E., Schrick, F. N., Lishman, A. W., Inskeep, E. K. and Butcher, R. L. 1993. Factors affecting fertility in the postpartum cow : role of the

oocyte and follicle in conception rate. *Biol. Reprod.*, 48 : 655-661.
7) Brogliatti, G. M. and Adams, G. P. 1996. Ultrasound-guided transvaginal oocyte collection in prepubertal calves. *Theriogenology*, 45 : 1163-1176.
8) Britt, J. H. 1992. Impacts of early postpartum metabolism on follicular development and fertility. *Proc. Am. Assoc. Bovine Pract.*, 24 : 39-43.
9) Campbell, B. K., Scaramuzzi, R. J. and Webb, R. 1995. Control of antral follicle development and selection in sheep and cattle. *J. Reprod. Fertil.*, 49 (*Suppl.*) : 335-350.
10) Canfield, R. W. and Butler, W. R. 1990. Energy balance and pulsatile LH secretion in early postpartum dairy cattle. *Dom. Anim. Endocrinol.*, 7 : 323-330.
11) Garcia, A. and Salaheddine, M. 1998. Effects of repeated ultrasound-guided transvaginal follicular aspiration on bovine oocyte recovery and subsequent follicular development. *Theriogenology*, 50 : 575-585.
12) Ginther, O. J., Kot, K., Kulick, L. J., Martin, S. and Wiltbank, M. C. 1996. Relationships between FSH and ovarian follicular waves during the last six months of pregnancy in cattle. *J. Reprod. Fertil.*, 108 : 271-279.
13) Gwazdauskas, F. C., Kendrick, K. W., Pryor, A. W. and Bailey, T. L. 2000. Impact of follicular aspiration on folliculogenesis as influenced by dietary energy and stage of lactation. *J. Dairy Sci.*, 83 : 1625-1634.
14) Hasler, J. F., Henderson, W. B., Hurtgen, P. J., Jin, Z. Q., McCauley, A. D., Mower, S. A., Neely, B., Shuley, L. S., Stokes, J. E. and Trimmer, S. A. 1995. Production, freezing and transfer of bovine IVF embryos and subsequent calving results. *Theriogenology*, 43 : 141-152.
15) Ireland, J. J., Mihm, M., Austin, E., Diskin, M. G. and Roche, J. F. 2000. Historical perspective of turnover of dominant follicles during the bovine estrous cycle : key concepts, studies, advancements, and terms. *J. Dairy Sci.*, 83 : 1648-1658.
16) Kendrick, K. W., Bailey, T. L., Garst, A. S., Pryor, A. W., Ahmadzadeh, A., Akers, R. M., Eyestone, W. E., Pearson, R. E. and Gwazdauskas, F. C. 1999. Effects of energy balance on hormones, ovarian activity and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. *J. Dairy Sci.*, 82 : 1731-1740.
17) Meintjes, M., Bellow, M. S., Broussard, J. R., Paul, J. B. and Godke, R. A. 1995. Transvaginal aspiration of oocytes from hormone-treated pregnant beef cattle for in vitro fertilization. *J. Anim. Sci.*, 73 : 967-974.
18) Nett, T. M., Cermak, D., Braden, T., Manns, J. and Niswender, G. 1988. Pituitary receptors for GnRH and estradiol, and pituitary content of gonadotropins in beef cows. II. Changes during the postpartum period. *Dom. Anim. Endocrinol.*, 5 : 81-89.
19) Looney, C. R., Lindsey, B. R., Gonseth, C. L. and Johnson, D. L. 1994. Commercial aspects of oocyte retrieval and *in vitro* fertilization (IVF) for embryo production in problem cows. *Theriogenology*, 41 : 67-72.
20) O'Callagan, D. and Boland, M. P. 1999. Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. *Anim. Sci.*, 68 : 299-314.
21) Pieterse, M. C., Kappen, K. A., Kruip, T. A. M. and Taverne, M. A. M. 1988. Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries. *Therio-*

genology, 30 : 751-762.

22) Rhodes, F. M., Clark, B. A., Hagemann, L. J. and Macmillan, K. L. 1997. Developmental competence of oocytes from postpartum dairy cows before and after resumption of oestrous cycles. *Proc. Aus. Soc. Reprod. Biol.*, 29 : 86 (abstract).

23) Sasamoto, Y., Sakaguchi, M., Katagiri, S., Yamada, Y. and Takahashi, Y. 2003. The effects of twisting and type of aspiration needle on the efficiency of transvaginal ultrasound-guided ovum pick-up in cattle. *J. Vet. Med. Sci.*, 65 : 1083-1086.

24) Savio, J. D., Boland, M. P., Hynes, N. and Roche, J. F. 1990. Resumption of follicular activity in the early post-partum period of dairy cows. *J. Reprod. Fertil.*, 88 : 569-579.

25) Sirois, J. and Fortune, J. E. 1990. Lengthening the bovine estrous cycle with low levels of exogenous progesterone : A model for studying ovarian follicular dominance. *Endocrinology*, 127 : 916-925.

26) Takahashi, Y., Hishinuma, M., Matsui, M., Tanaka, H. and Kanagawa, H. 1996. Development of *in vitro* matured/fertilized bovine embryos in a chemically defined medium : influence of oxygen concentration in the gas atmosphere. *J. Vet. Med. Sci.*, 58 : 897-902.

27) Tohei, A., Shi, F. X., Ozawa, M., Imai, K., Takahashi, H., Shimohira, I., Kojima, T., Watanabe, G. and Taya, K. 2001. Dynamic changes in plasma concentrations of gonadotropins, inhibin, estradiol-17β and progesterone in cows with ultrasound-guided follicular aspiration. *J. Vet. Med. Sci.*, 63 : 45-50.

28) Walters, A. H., Bailey, T. L., Pearson, R. E. and Gwazdauskas, F. C. 2002. Parity-related changes in bovine follicle and oocyte populations, oocyte quality and hormones to 90 days postpartum. *J. Dairy Sci.*, 85 : 824-832.

29) Walters, A. H., Pryor, A. W., Bailey, T. L., Pearson, R. E. and Gwazdauskas, F. C. 2002. Milk yield, energy balance, hormone, follicular and oocyte measures in early and mid-lactation Holstein cows. *Theriogenology*, 57 : 949-961.

30) Webb, R., Nicholas, B., Gong, J. G., Campbell, B. K., Gutierrez, C. G., Garverick, H. A. and Armstrong, D. G. 2003. Mechanisms regulating follicular development and selection of the dominant follicle. *Reproduction*, 61 (*Suppl.*) : 71-90.

31) Xu, Z. Z., Garverick, H. A., Smith, G. W., Smith, M. F., Hamilton, S. A. and Youngquist, R. S. 1995. Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during the first follicular wave. *Biol. Reprod.*, 53 : 951-957.