Control of Corpus Luteum Function for Shortening the Conception Interval of Dairy Cows

Seiji YAMAMOTO*, Hirotada TSUJII and Kazuyosi HASHIZUME**

Faculty of Agriculture, Shinshu University

Summary

The present study was undertaken to evaluate the shortening of conception interval by artificially controlling the function of corpus luteum. A commercial solution of polyvinyl pyrolidone and iodine was infused into the uterine cavity on d 10 postpartum, subsequently using human chorionic gonadotropin, $PGF_{2\alpha}$, or GnRH to induce estrus 30 to 40 d postpartum. Cows in the control group, showing normal signs of estrus approximately 35 d postpartum were used for artificial insemination without any other artificial treatment. Only 5 of 124 cows showed signs of estrus at 35 d after parturition, but these cows failed to conceive after artificial insemination. The follicular development and subsequent estrus signs were found 1/3, 2/2, 5/5, 3/4 and 2/4 for the treatment of polyvinyl pyroidone and iodine only, hCG+$PGF_{2\alpha}$, $PGF_{2\alpha}$, GnRH and $PGF_{2\alpha}$+GnRH, respectively. Subsequently, 13 of 18 cows showed signs of estrus and ovulation. Pregnancy rates were 50 and 60% for the cows treated with hCG+$PGF_{2\alpha}$ and $PGF_{2\alpha}$ alone, respectively; and the total treated conception cows were 22% within d 33.8 (mean) postpartum. Cows not conceived by d 35 postpartum usually (12 of 14) conceived within d 60 postpartum. The remaining 2 cows had ovarian cysts. Treatment with $PGF_{2\alpha}$ alone or with hCG followed by the injection of PVP+I artificially adjusted the function of corpus luteum, took place ovulation, and occurred conception within d 35 postpartum.

(Jour. Fac. Agric. Shinshu Univ. 33 : 61-70, 1996)

Key words : polyvinyl pyroidone and iodine, corpus luteum, human chorionic gonadotropin , prostaglandin $F_{2\alpha}$

Introduction

Shorter reproductive intervals of dairy cows are considered to contribute to the increased milk production, synchronization of estrus, and enhancement of conception rates. In our previous studies[28], we observed that estrus recurred at d 32.3 (mean), and

Received September 30, 1996

* Veterinary Practitioner, Ina, Nagano,

** National Institute of Animal Industry, Tsukuba, Ibara

artificial insemination was possible by d 35 for 3 of the 6 cows when they were infused by polyvinyl pyrolidone and iodine (PVP+I) at d 10 postpartum. This interval from parturition to artificial insemination was significantly shorter, than that of the 60 control cows (90.0 ±32.7 d). Intrauterine infusion of PVP+I induced the early return of reproductive function, the onset of estrus, the development of corpus luteum, the recurrence of estrus after luteal regression, and the realization of artificial insemination occured within 40 to 60 d post parturition.

The postpartal onset of estrus usually appears after short-term luteinization[11,14,15]. Early weaning of anestrus cows after parturition leads to the appearance of estrus behavior after approximately 4 d[2]. Butcher et al.[5] reported that the uterus of some postpartum cows with a short-lived corpus luteum was capable of maintaining gestation. These phenomena are usually applicable to the initial formation of corpus luteum in dairy cows [16,17,18,19]. Breuel et al.[4] reported that 88% of ova collected from postpartum estrus cows attained normal growth, but were not fertilized when transplanted in postpartum estrous cows. They indicated that the uterine condition, namely the environment surrounding the ova was not responsible for the failure to fertilize[5]. The rates of conception and estrus onset could be enhanced if postpartum cows were treated with norgestomet, a synthetic progesterone, within 12 d prior to the onset of estrus[4,5].

The present study was undertaken to evaluate the shortening of conception interval by artificially controlling the function of corpus luteum. A commercial preparation of PVP+I was infused into the uterine cavity on d 10 of postpartum and subsequently using human chorionic gonadotropin (hCG), $PGF_{2\alpha}$, or GnRH to induce estrus in between d 30 to 40 postpartum.

Materials and Methods

We used 124 multiparous Holstein cows as the control group and 18 multiparous Holstein cows in experimental group. Cows were fed rice straw, hay, silage, and composite fodder and were reared in confinement. The past deliveries were confirmed to be normal for both groups of cows. In the control group, the cows showing signs of estrus on about d 35 postparturition were used for artificial insemination without other artificial treatments.

The experimental cows were divided into five sub-groups; polyvinyl pyroidone and iodine only, hCG+$PGF_{2\alpha}$, $PGF_{2\alpha}$, GnRH and $PGF_{2\alpha}$+GnRH, with 3, 2, 5, 4 and 4 cows, respectively. In all experimental animals, on d 10 postpartum, 50 ml of PVP+I (Fujita Seiyaku Co., Led. Tokyo, Japan) was infused into the uterine cervix, and ovarian changes were observed by rectal palpation at 5-d intervals. Three of them were maintained as sub-group one. The cows contained a small soft corpus luteum were considered as having low activation corpus luteum ovary. To activate these types of corpus luteum, 3000 IU

of hCG (Sankyo Co. Ltd. Tokyo, Japan) with 12 mg/ml of $PGF_{2\alpha}$ (Panacelan F Solution, Daiichi Pharmaceutical Co., Ltd. Tokyo, Japan) was infused intramuscularly in sub-group two. For luteolysis and estrus return, 12 mg/ml of $PGF_{2\alpha}$ (Panacelan F Solution, Daiichi Pharmaceutical Co., Ltd. Tokyo, Japan) was infused intramuscularly in third sub-group. The cows with undeveloped follicle in ovary, 5 ml of GnRH (Shionogi Co.Ltd. Tokyo, Japan) was infused intramuscularly in sub-group four. In last sub-group, 12 mg/ml of $PGF_{2\alpha}$ (Panacelan F Solution, Daiichi Pharmaceutical Co., Ltd. Tokyo, Japan) with 5 ml of GnRH (Shionogi Co.Ltd. Tokyo, Japan) was infused intramuscularly. The degree of uterine recovery was monitored by rectal palpation technique, involving the degree of thickness, shrinking, lochia of uterine body, and the size and hardness of corpus luteum. The cows occurring normally corpus luteum luteolysis and subsequently follicular development were used in these experiments. The cows ovulated and showed signs of estrus were inseminated artificially with 0.5 ml of frozen semen by administration into the uterine horn and pregnancy was confirmed by rectal examination on d 60 after artificial insemination. The progesterone concentrations of milk were determined by enzyme immuno assay technique [25].

Results

Only 5 of 124 control cows showed signs of estrus on d 35 postpartum. The progesterone concentrations of milk was used as an indication of estrus. Although the uterus, uterine horn and body appeared to be normal, these 5 cows failed to conceive after artificial insemination (Table 1).

Similarly, in treatment PVP+I, only 1 of 3 cows showed signs of estrus and failed to conceive. Cows receiving hCG+$PGF_{2\alpha}$ and $PGF_{2\alpha}$ followed by the infusion of PVP+I showed sign of estrus and ovulation and subsequently conception observed in 50 and 60%, respectively. Treatment with GnRH or $PGF_{2\alpha}$ with GnRH produced sign of estrus in 75 and 50% of cows, respectively, but both these group failed to conceive (Table 1).

Without additional treatment with PVP+I, signs of estrus were observed, and the

Table 1. Effect of polyvinyl pyrolidone iodine and hormone treatment on estrous and ovulation in dairy cows

Treatment	Rate of estrous and ovulation	Rate of pregnacy
Control (no treatment)	5/124(4 %)	0(0%)
PVP-I	1/3(33.3%)	0(0%)
PVP-I+hCG+$PGF_{2\alpha}$	2/2(100 %)	1/2(50%)
PVP-I+$PGF_{2\alpha}$	5/5(100 %)	3/5(60%)
PVP-I+GnRH	3/4(75 %)	0(0%)
PVP-I+$PGF_{2\alpha}$+GnRH	2/4(50 %)	0(0%)

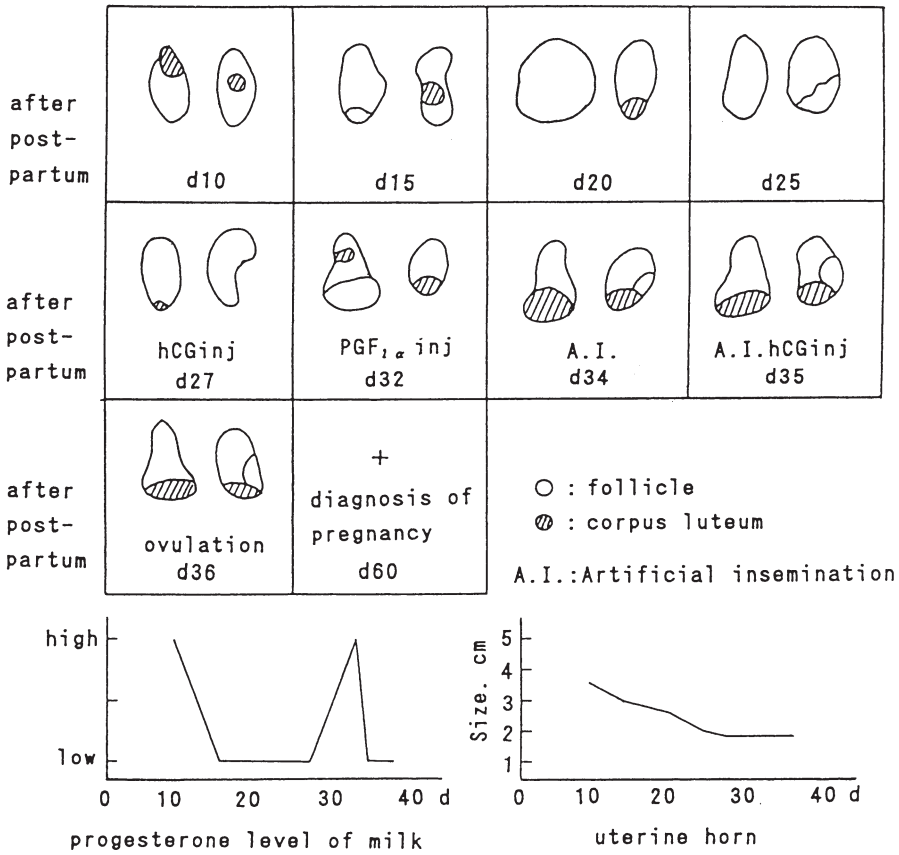

Fig. 1 Changes of the morphology of ovary and uterine horn and progesterone level of milk when treated with hCG+$PGF_{2\alpha}$ after injection of iodine solution in the uterus.

follicular development occurred on d 45 postpartum. Then the uterine walls were enlarged and uterine mucus was in semitranslucent condition. However, the artificial insemination in these cases was not successful. The ovarian changes caused by infusion of hCG+$PGF_{2\alpha}$ are presented in Figure 1. The administration of hCG initiated luteolysis, softened the follicle, but did not occur the luteinization on d 27. Administration of $PGF_{2\alpha}$ regressed the corpus luteum on d 32. Estrus occurred, and follicular development was observed on d 35. Subsequently, conception occurred by artificial insemination of those cows.

Similarly, changes of ovarian stage caused by administration of $PGF_{2\alpha}$ are shown in Figure 2. The cows showing the existence of a corpus lutuem by d 25 postpartum, had corpus luteum regressed with $PGF_{2\alpha}$ and 7 d later showed estrus. Follicular development was observed on d 30 postpartum, and conception occurred after artificial insemination.

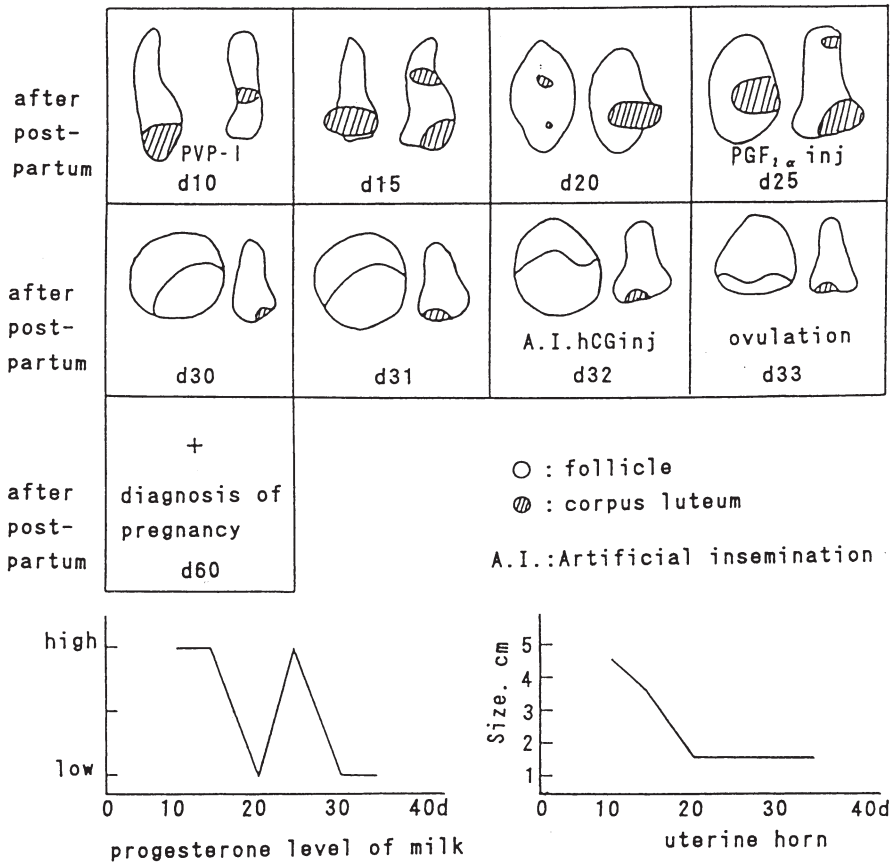

Fig. 2 Changes of the morphology of ovary and uterine horn and progesterone level of milk when treated with $PGF_{2\alpha}$ after injection of iodine solution in uterus.

The cows treated with GnRH showed the existence of follicle and signs of estrus were confirmed on d 29 postpartum, and ovulation occurred simultaneously. Conception, however, had not occurred on d 30 postpartum. The cow that was infused with $PGF_{2\alpha}$+GnRH, showed corpus lutuem regression on d 28 postpartum, and GnRH was infused on d 31 postpartum to develop follicle. The results of artificial insemination in these cases was failure to conceive.

Thirteen of 18 cows showed signs of estrus and ovulation. Pregnancy rates were 50 and 60% for the treatments with hCG+$PGF_{2\alpha}$ and $PGF_{2\alpha}$ alone, respectively; total conception rates for treated cows were 4 of 18 within d 33.8 (mean) postpartum. Most cows that had not conceived by 35 d conceived (12 of 14), within d 60 postpartum (Table 1). The remaining 2 cows had the ovarian cysts and did not conceive.

Discussion

Five of 124 control cows showed signs of estrus during d 35 postpartum, but they failed to conceive after artificial insemination. One of the main reasons for conception failure may be incomplete involution and swelling of the uterus and the semitranslucent uterine mucus. However, the cows with incomplete uterine involution were infused with PVP+I. Then, the uterine mucus became transparent, and a high degree of uterine wall shrinkage was observed. The degree of shrinking of the uterine wall and the characteristics of the uterine mucus may be important factors for conception. The insufficient preparation of uterine mucus may be a cause of early embryonic death.

After parturition, estrus cycle of cows become shorter[12,17]. For beef cattle, about 80% anestrus was evident within d 10 postweaning; after first estrus, progesterone concentrations in serum increased within the shorter reproductive cycle, which is within d 7 to 12[16,19]. Progesterone in serum rises to high concentrations before the first postpartum estrus[27]. If blood progesterone did not rise to exact level before estrus, ovulation did not occur, even though signs of estrus had been observed[17,18]. Practically all cows that had low serum progesterone before first estrus after post-weaning were anestrus cows. Synthetic progesterone, such as norgestomet was implanted subcutaneously or administered as 100 mg of progesterone twice daily from preweaning induced estrus postpartum[4,17]. The cows in which norgestomet were implanted subcutaneously for d 9 pre-weaning, occurred subsequent first and second estrus within d 4 and 8 to 10 post-weaning[4]. The ability to obtain normal luteal phase and 33% conception were also obtained with norgestomet treatment during the pre-weaning phase[17]. In our present study, conception rate was 57.1% with $PGF_{2\alpha}$ or with $PGF_{2\alpha}$ and hCG.

The synthetic progesterone had the effective response to estrus and fertilization. If the progesterone administration started during the last stage of estrus cycle and continued up to 12 d, then estrus become late, and finally the rate of conception declined[1,21,22]. Short progesterone treatment with higher concentration is necessary for luteolysis to occur and to obtain a high conception rate[26]. For cows during the estrus cycle, luteolysis took place, but due to estrus atresia of the ovary one to two dominant follicle usually did not ovulate[7,9,23]. By changing the concentration of corpus luteum hormone it is possible to change the state of the dominant follicle[3]. A low progesterone concentration (1 to 2 ng/ml) could extend the life of dominant follicle[24]. Administration of norgestomet, if progesterone concentration was usually low, increased the LH surge concentration of estradiol[10,20]. As a result, progesterone could have an effect on ovulation, fertilization, and early follicular development, but early embryonic death also occurred[4]. Mihn et al[13]. suggested that the dominant follicle, which spent >10 d for ovulation would not be suitable for conception, and the suitable time for dominance

follicle is usually <8 d. In the present study, we observed the positive effect of $PGF_{2\alpha}$ and hCG, but the function of hormone secreted from corpus luteum, LH, is still unknown.

Zimbelman and Smith[29] obtained high conception rate from ovariectomized cows by daily feeding of 1 mg/d of melengestrol acetate (MGA) starting from d 4 after estrus. Inskeep and Baker[8] obtained 33.3% conception rate by the administration of MGA (4 mg/d) on uterine transplanted ovariectomized cows after induced estrous postpartum. Butcher et al[5]. reported 28% conception rate with sequential administration of progesterone (100 mg/cow, twice a day) at the early stage of luteolysis using d 7 postpartum cows and frozen-thawed embryo. Furthermore, in postpartum cows at pre-estrus stage continuous administration of progesterone resulted in 58% conception rate. By the same method Breuel et al[4]. also obtained 50% conception rate. From these results, the administration of corpus luteum hormone induced estrus in postpartum cows and maintained pregnancy even without the existence of the ovary.

In the present study, we infused hCG to complete corpus luteum formation at pre-estrus stage. Human chorionic gonadotropin hormone was selected to enhance the function of corpus luteum. In fact, we knew that the infusion of progesterone twice daily is not suitable in the experiment. The hCG were clinically used to enhance the function of corpus luteum in embryo transfer technique. However, hCG is not totally pure; therefore, administration of hCG in high concentration may increase the function of FSH may be due to the presence of some follicle stimulating hormone in it. Chupin et al[6]. administered synthetic progesterone and protaglandins to 620 individual of anestrus beef cows and obtained offspring from 28.5% of those. The $PGF_{2\alpha}$ was functionally effective without any adverse effect; corpus luteum was regressed, and follicular development occurred. This subsequent follicular development had a positive effect on conception.

In another trial, one Japanese beef type cow was mated with a bull when signs of estrus had appeared after PVP+I administration, resulting in pregnancy. This treatment was effective, but in practice it would not be recommended because uterine involution occurred partially on d 35 postpartum. In our experiment we used 0.5 ml of semen for each insemination, but this volume may be small, and the possibility that the semen passed to oviduct may be usually little. We suggest that the volume of semen and the number of sperm to be used in the artificial insemination needs to be increased in future in case of postpartum cow.

In conclusion, we may stated that intrauterine infusion of PVP+I induces the early return of reproductive function, the onset of estrus, the development of corpus luteum and the recurrence of estrus after luteal regression. However, the results of subsequent artificial insemination was not successful. The treatment of $PGF_{2\alpha}$ and with hCG, followed by the infusion of PVP+I artificially adjusted the function of corpus luteum and ovulation took place followed by conception within d 35 postpartum.

References

1) Beal, W. E., J. R. Chenault, M. L. Day, and L. R. Corah. 1988. Variation in conception rates following synchronization of estrus with melengestrol acetate and prostaglandin $F_{-2\alpha}$. J. Anim. Sci. 66:599.

2) Bellows, R. A., R. E. Short, J. J. Urick, and O. F. Pahnish. 1974. Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiples or singles. J. Anim. Sci. 39:589.

3) Bergfelt, D.R., J. P. Kastelic, and O. J. Ginther. 1991. Continued periodic emergence of follicular waves in non-bred progesterone-treated heifers. Anim. Reprod. Sci. 24:193.

4) Breuel, K. F., P. E. Lewis, F. N. Schrick, A. W. Lishman, E. K. Inskeep, and B. L. Butcher. 1993. Factors affecting fertility in the postpartum cow : Role of the oocyte and follicle in conception rate. Biol. Reprod. 48:655.

5) Butcher, R. L., J. E. Reber, A. W. Lishman, K. F. Breuel, F. N. Schrick, J. C. Spitzer, and E. K. Inskeep. 1992. Maintenance of pregnancy in postpartum beef cows having short-lived corpora lutea. J. Anim. Sci. 70:3831.

6) Chupin, D., J. Pelot, and P. Mauleon. 1977. Control of oestrus and ovulation in dairy cows. Theriogenology 7:339.

7) Ginther, O. J., I. Knopf, and J. P. Kastelic. 1989. Composition and characteristics of follicular waves during the bovine oestrous cycle. Anim. Reprod. Sci. 20:187.

8) Inskeep, E. K., and R. D. Baker. 1985. Successful transfer of bovine embryos into ovariectomized recipients. J. Anim. Sci. 61 (Suppl 1) :409. (Abstr.).

9) Ireland, J. J. and J. F. Roche. 1987. Hypotheses regarding development of dominant follicles during bovine oestrous cycles. Page 1, in Follicular Growth and Ovulation Rate in Farm Animals. J. F. Roche. and D O'Callaghan, ed. Martinus Nijhoff. The Hague, The Netherlands.

10) Kojima, N., T. T. Stumpf, A. S. Cupp, L. A. Werth, M. S. Roberson, M. W. Wolfe, R. J. Kittok, and J. E. Kinder. 1992. Exogenous progesterone and progestins as used in estrous synchrony regimens do not mimic the corpus luteum in regulation of luteinizing hormone and 17β-estradiol in circulation of cows. Biol. Reprod. 47:1009.

11) LaVoie, Han D. K., Foster, D. B., and E. L. Moody. 1981. Suckling effect on estrus and blood plasma progesterone in postpartum beef cows. J. Anim. Sci. 52:802.

12) Macmillan, K. L., and J. D. Watson. 1971. Short estrous cycles in New Zealand dairy cattle. J. Dairy Sci. 54:1526.

13) Mihm. M., A. Baguisi, M. P. Boland and J. F. Roche. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J. Reprod. Fert., 102: 123.

14) Morrow, D. A., S. J. Roberts, and K. McEntee. 1969. A review of postpartum ovarian activity and involution of the uterus and cervix in cattle. Cornell Vet. 59:134.

15) Morrow, D. A., S. J. Roberts, K. McEntee, and H. G. Gray. 1966. Postpartum ovarian

activity and uterine involution in dairy cattle. J. Am. Vet. Med. Assoc. 149:1596.

16) Odde, K. G., H. S. Ward, G. H. Kiracofe, R. M. Mckee, and R. J. Kittok. 1980. Short estrous cycles and associated serum progesterone levels in beef cows. Theriogenology 14:105.

17) Ramirez-Godinez, J. A., G. H. Kiracofe, D. L. Carnahan, M. F. Spire, K. B. Beeman, J. S. Stevenson, and R. R. Schalles. 1982b. Evidence for ovulation and fertilization in beef cows with short estrous cycles. Theriogenology 17:409.

18) Ramirez-Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles, and R. J. Kittok. 1981. Reducing the incidence of short estrous cycles in beef cows with norgesteromet. Theriogenology 15:613.

19) Ramirez-Godinez, J. A., G. H. Kiracofe, R. R. Schalles, and G. D. Niswender. 1982a. Endocrine patterns in the postpartum beef cow associated with weaning : comparison of the short and subsequent normal cycles. J. Anim. Sci. 55:153.

20) Roberson, M. S., M. W. Wolfe, T. T. Stumpf, R. J. Kittok, and J. E. Kinder. 1989. Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. Biol. Reprod. 41:997.

21) Roche, J. F. 1974. Synchronization of oestrus in heifers with implants of progesterone. J. Reprod. Fertil. 41:337.

22) Roche, J. F. 1978. Control of oestrus in cattle using progesterone coils. Anim. Reprod. Sci. 1:145

23) Sirois, J., and J. E. Fortune. 1988. Ovarian follicular dynamics during the estrous cycle monitored by real-time ultrasonography. Biol. Reprod. 39:308.

24) Sirois, J., and J. E. Fortune. 1990. Lengthening of the bovine estrous cycle with two levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 127:916.

25) Tsujii, H., S. Yamamoto, K. Kojima, and K. Hashizume. 1988. The use of simple milk test kit for the diagnosis of pregnancy in cows. Jpn. J. Anim. Reprod. 34:84.

26) Wehrman, M. E., M. S. Roberson, A. S. Cupp, F. N. Kojima, T. T. Stumpf, L. A. Werth, M. W. Wolfe, R. J. Kittok, and J. E. Kinder. 1993. Increasing exogenous progenterone during synchronization of estrus decreases endogenous 17β-estradiol and increases conception in cows. Biol. Reprod. 49:214.

27) Wettemann,R. P., 1980. Postpartum endocrine function of cattle, sheep and swine. XIV Biennial Symp. on Animal Repro. J. Anim. Sci. 51(Suppl 2) : 6.

28) Yamamoto, S., Tsujii, H., Hashizume, K. and Sugawara, S. 1996. Effect of intrauterine infusion of polyvinyl pyrolidone iodine on early return of reproductive function in postpartum dairy cows. Tohoku J. Agri. Res., 47 (1-2), 19-27.

29) Zimbelman, R. G., and L. W. Smith. 1966. Maintenance of pregnancy in ovariectomized heifers with melengestrol acetate. J. Anim. Sci. 25:207.

乳牛の黄体制御による分娩後の受胎早期化

山本静二*・辻井弘忠・橋爪一善**
信州大学農学部生物資源開発学
*開業医，長野県伊那市
**農林水産省畜産試験場

要　　約

乳牛の分娩後人為的に黄体を制御することによって分娩後の受胎を早期化することを検証した。商品化されているポリビニールピロリドン・ヨード液（PVP-I）を分娩後10日目の子宮内に注入し，その後，hCG（ヒト絨毛性性腺刺激ホルモン），$PGF_{2\alpha}$，GnRH を用いて分娩後30-40日以内に発情を誘発した。対照区の牛は，分娩後35日以内に発情を示した牛で，人為的処理を行わず人工授精を行った。その結果124頭の内５頭が分娩後発情を示したが受胎しなかった。一方，人為的処理群においてPVP-Iのみでは１/３，PVP-I投与後hCG＋$PGF_{2\alpha}$区で２/２，PVP-I投与後$PGF_{2\alpha}$区で５/５，PVP-I投与後GnRH区で３/４，PVP-I投与後$PGF_{2\alpha}$＋GnRH区で２/４が卵胞の発達と発情がみられた。その18頭の内13頭が発情と排卵がみられた。人工授精の結果hCG＋$PGF_{2\alpha}$と$PGF_{2\alpha}$区では各々50と60％妊娠し，総処理牛での平均分娩後33.8日に22％が受胎した。処理牛の中で35日までに発情が来なかった14頭の内12頭は分娩後60日までに受胎した。残る２頭は卵巣嚢腫で不受胎であった。分娩後PVP-I子宮注入後の$PGF_{2\alpha}$の単一またはhCG＋$PGF_{2\alpha}$投与による黄体機能を制御は分娩35日までに排卵および受胎を可能にした。