Luteal Hypoplasia and Induced Ovulation in Cows

Yohji OHNAMI, Motohiro KIKUCHI, and Hideo ONUMA

Department of Veterinary Reproduction, School of Veterinary Medicine and Animal Science, Kitasato University, Towada, Aomori 034, Japan

(Received 28 October 1985/Accepted 26 May 1986)

ABSTRACT. A relationship between luteal hypoplasia and ovulation was analyzed in cows. Adult cows of the luteal phase were injected with gonadotropin releasing hormone analogue (GnRH-A) 16 hr (group I of 4 head), 32 hr (group II of 4 head), or 48 hr (group III of 3 head) after injection with prostaglandin F$_{2\alpha}$-analogue (PGF$_{2\alpha}$-A), and then examined for the occurrence of luteal hypoplasia. Ovulation took place at 32–46 hr (37.5 hr on the average) in group I and 9–33 hr (20.0 hr on the average) in group II after injection with GnRH-A. Unsatisfactory luteinization and substantial shortening in estrous cycle were observed in all the cows of groups I and II. In group III, ovulation took place 12–32 hr (24.7 hr on the average) after injection with GnRH-A, and luteinization was satisfactory in one, but the corpora lutea formed were small in the other two cows. All the cows, except one in each of groups II and III were examined for changes in the plasma progesterone level after the induced ovulation. Low progesterone levels were observed in groups I and II, while in group III, the levels took almost the same course of changes as the normal estrous cycle, indicating the presence of a satisfactory luteinization. However, temporary increase was observed in another cow in this group. It is concluded that luteinization became unsatisfactory when ovulation was forced to take place in cows while the ovarian follicles were still developing.—KEY WORDS: cow, induced ovulation, luteal hypoplasia.

In 1959, Armstrong and Hansel [1] successively administered oxytocin to cows at the beginning of the estrous cycle. The administration disturbed their luteinizations so seriously that the corpora lutea degenerated early and the estrous cycle was reduced. In this manner, they succeeded in experimentally producing short-lived corpora lutea. Furthermore, they observed that the corpora lutea were prevented from early degeneration by oxytocin administration when bovine luteinizing hormone (LH), bovine hypophyseal extract, or human chorionic gonadotropin (HCG) had been simultaneously administered to these cows. To explain their observations, they presumed that oxytocin might have been secreted to inhibit the secretion of LH from the anterior lobe of the hypophysis [3]. However, no early degeneration of the corpus luteum was induced by oxytocin in hysterectomized cows [2]. Recently, Milvae and Hansel [13] observed that oxytocin administration caused an increase in the level of the prostaglandin F (PGF) group without changes in the blood LH concentration. The observation clearly indicated that oxytocin might not directly inhibit the hypophyseal function. It was subsequently elucidated that some early formed corpora lutea were degenerated by the action of oxytocin exerted through the uterus. Accordingly, in the experiment conducted by Armstrong and Hansel [1], some short-lived corpora lutea were not produced by any disturbance in the luteinization mechanism.

In the previous experiment, we made an attempt to stimulate the estrus and ovulation cycle in cows after parturition by gonadotropin releasing hormone analogue (GnRH-A). We observed that small corpora lutea were formed and degenerated.
before long when ovulation took place from a small ovarian follicle in the stage of development. The results indicate that there might be a relationship between the maturity of an ovarian follicle and luteinization. In the present investigation, an attempt was made to experimentally produce luteal hypoplasia by controlling the maturity of an ovarian follicle at the time of ovulation.

MATERIALS AND METHODS

*Experimental cows:* A total of 10 cows were employed; one was employed twice. They consisted of 4 Holsteins, 2 Jerseys, and 4 Japanese Shorthorns. Their weights ranged from 428–739 kg and averaged 559.9 kg.

*Induction of ovulation:* Experimental cows, in which the preceding estrous cycles were normal, were intramuscularly injected in the buttocks or shoulder with 0.8–1.0 mg of PGF<sub>2α</sub>-analogue, ONO-1052 (PGF<sub>2α</sub>-A, Ono Pharmaceutical Co., Ltd., Osaka) 10 days after ovulation (the day of ovulation being expressed as day 0). The injection was expected to degenerate in the corpora lutea and to develop follicles for the following ovulation. When the rectal examination at the time of PGF<sub>2α</sub>-A injection revealed the presence of a large follicle in addition to a corpus luteum of functional state, the subjects were excluded from the experiment. Ovulation was induced by using GnRH-A, TAP-031 (Takeda Chemical Industries, Ltd., Osaka). Each cow was intramuscularly injected with 200 or 1,000 μg of this agent by the method of Kittok *et al.* [8]. The dose was divided into two equal portions and injected at 60 min-interval. Three experimental groups, I, II, and III, were set up and injected with GnRH-A at 16, 32, and 48 hr, respectively, after injection with PGF<sub>2α</sub>-A. Since one of the 10 cows was employed twice, groups I, II, and III consisted of 4, 4, and 3 cows, respectively.

*Estrous and rectal examinations:* Estrous examination was performed at 8 hr-intervals in a period from PGF<sub>2α</sub>-A injection to GnRH-A injection, at 6 hr-intervals in a period from GnRH-A injection to ovulation, and twice a day (8:30 a.m. and 4:30 p.m.) during the other period. Each examination took about 30 min.

Rectal examination was carried out on the first and second day of estrus and on the 5th and 10th days after ovulation prior to PGF<sub>2α</sub>-A injection. It was conducted at 8 hr-intervals between PGF<sub>2α</sub>-A and GnRH-A injections and at 3–6 hr-intervals between GnRH-A injection and induced ovulation. After the induced ovulation was confirmed, it was performed every day until the following day of ovulation.

*Blood sampling and estimation of plasma progesterone level:* Blood samples were collected at 24 hr-intervals between induced ovulation and the beginning of the following estrous period, and at other periods designated in the text. Blood sampling was not performed with one cow in each of groups II and III. Each sample was collected from the jugular vein into a tube containing the anticoagulant, heparin (Novo Company, Denmark). The blood samples were placed immediately on crushed ice followed by centrifugation at 3,000 rpm for 20 min at 5°C to separate the blood plasma. The separated plasma was stored at −20°C. The plasma progesterone level was estimated by the radioimmunoassay method of Makino *et al.* [11, 12], except that fractionation and purification by chromatography were omitted in this method.

*Statistical analysis:* The experimental results were analysed by the student's t-test.

RESULTS

1. The ovulation and luteinization after injection with the gonadotropin releasing hormone analogue.
Table 1. Ovulation and luteinization in cows after injection with gonadotropin releasing hormone analogue (GnRH-A)

<table>
<thead>
<tr>
<th>Group</th>
<th>GnRH-A (µg)</th>
<th>Experimental cow</th>
<th>Occurrence of estrus&lt;sup&gt;3)&lt;/sup&gt;</th>
<th>Ovulation induced</th>
<th>Luteinization</th>
<th>Subsequent estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time after injection with GnRH-A(hr)</td>
<td>Time after injection with PGF&lt;sub&gt;2α&lt;/sub&gt;-A(hr)</td>
<td>Condition</td>
<td>Maximum longer diameter (cm)</td>
</tr>
<tr>
<td>I</td>
<td>200</td>
<td>H-1</td>
<td>36</td>
<td>52</td>
<td>No good&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-71</td>
<td>36</td>
<td>52</td>
<td>No good&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>S-44</td>
<td>32</td>
<td>48</td>
<td>No good&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H-5</td>
<td>46</td>
<td>62</td>
<td>No good&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>II</td>
<td>200</td>
<td>S-3</td>
<td>19</td>
<td>51</td>
<td>No good&lt;sup&gt;c)&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H-53</td>
<td>19</td>
<td>51</td>
<td>No good&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>S-78</td>
<td>9</td>
<td>41</td>
<td>No good&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J-1</td>
<td>33</td>
<td>65</td>
<td>No good&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>III</td>
<td>200</td>
<td>J-1</td>
<td>±&lt;sup&gt;e)&lt;/sup&gt;</td>
<td>32</td>
<td>No good&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J-2</td>
<td>±&lt;sup&gt;e)&lt;/sup&gt;</td>
<td>30</td>
<td>Normal</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H-2</td>
<td>—</td>
<td>12</td>
<td>No good&lt;sup&gt;d)&lt;/sup&gt;</td>
<td>1.5</td>
</tr>
</tbody>
</table>

a) Interval between injection with PGF<sub>2α</sub>-A and ovulation.
b) No corpus luteum palpable at the site of ovulation.
c) Cystic corpus luteum formed.
d) Small corpus luteum formed.
e) Desire to mount at the time of injection with GnRH-A, but not after injection.
f) Estrus of short duration 13 hr after injection with GnRH-A.
g) No follicles developed well after degeneration of the corpus luteum. Anestrus persisted for about 30 days.
two cows injected with 1,000 μg, ovulation was induced at 32 and 46 hr, respectively. In other words, ovulation was induced in the four cows on the average of 37.5 hr after injection. No estrous behaviors were noticed in any of the cows during the period from PGF$_{2α}$-A injection to induced ovulation. None of the cows of group I had satisfactory luteinization after ovulation was induced. One of the two cows which were injected with 200 μg, had no palpable corpus luteum at the site of ovulation, while the other had a cystic corpus luteum. Two cows injected with 1,000 μg had small corpora lutea, 1.0 and 1.5 cm in longer diameters 4 days after the ovulation, and these corpora lutea later reduced in size. The subsequent estrus was noticed 6-8 days after the induced ovulation. Most of the cows showed abnormally short estrous cycles.

Ovulation was induced in the two cows in group II at 19 hr after they were injected with 200 μg of GnRH-A. In the other two cows, which had been injected with 1,000 μg of GnRH-A, ovulation was induced at 9 and 33 hr after the injection. However, silent estrus was observed in all the cows. Ovulation was induced on the average 20.0 hr after injection with GnRH-A. This interval was significantly shorter (P<0.05) than that of group I. Therefore, the interval between injection with PGF$_{2α}$-A and ovulation was almost the same in groups I and II (P>0.05).

Luteinization after induced ovulation was also abnormal in group II. One of the two cows that were injected with 200 μg of GnRH-A, had a small corpus luteum 4 days after the induced ovulation. It was 0.8 cm in the longer diameter and reduced later in size. The other one showed a cystic corpus luteum. Of the two cows injected with 1,000 μg of GnRH-A in group II, one possessed a small corpus luteum of 1.0 cm in the longer diameter 3 days after induced ovulation followed by its eventual degeneration, while the other cow had no palpable corpus luteum at the site of ovulation. Normal estrus was observed in one of these two cows 10 days after induced ovulation. The other cows in group II showed abnormal estrus 4-6 days after induced ovulation.

In group III, ovulation was induced in the three cows at 12, 30, and 32 hr after injection with 200 μg of GnRH-A. One cow exhibited a mounting behavior and another cow showed a behavior of being mounted around at the time of injection with GnRH-A. In this group, ovulation was induced on the average of 24.7 hr after injection with GnRH-A. There was no significant difference in this time interval among the three groups. Nevertheless, the time intervals between injection with PGF$_{2α}$-A and ovulation was significantly longer (P<0.05) in group III than those in groups I or II.

In the luteinization after the induced ovulation, the cow, which had shown a behavior of being mounted, had a normal corpus luteum of 3.0 cm in the longer diameter in the functional state. It showed estrus 24 days after the induced ovulation. The other two cows belonging to group III possessed small corpora lutea at first. In one of them, the corpus luteum developed to be 1.5 cm in longer diameter 9 days after induced ovulation, and remained palpable when rectal examination was performed even 7 days later. In the other cow, it developed to 2.0 cm in the longer diameter 7 days after induced ovulation followed by a decrease in size to 1.5 cm 6 days later. Of these two cows, one presented a normal estrus 19 days after the induced ovulation. The other showed poor follicular growth after the degeneration of the small corpus luteum and remained anestrous for about 30 days.

2. Plasma progesterone level after induced ovulation by injection with gonadotropin releasing hormone analogue.

Figure 1 shows plasma progesterone
levels in the four cows of group I. Two (H-1 and S-71) were injected with 200 μg and the other two (H-5 and S-44) with 1,000 μg of GnRH-A. In the former group, progesterone levels remained low until 6 days after induced ovulation, when abnormal estrus was noticed. In cow H-1, it ranged from 0.5 to 1.8 ng/ml and was 1.0 ng/ml on the average. In cow S-71 which had a cystic corpus luteum, it ranged from 0.8 to 1.1 ng/ml and was 1.0 ng/ml on the average. In the latter group, the level tended to rise slowly after the induced ovulation. It reached 3.9 ng/ml in cow S-44 and 2.8 ng/ml in cow
H-5, 4 and 6 days, respectively, after the induced ovulation, and then began to fall.

Figure 2 shows the plasma progesterone level in three cows of group II. Two (S-3 and H-53) were injected with 200 μg and the other one (S-78) was with 1,000 μg of GnRH-A. In cow S-3, the level remained as low as 0.6–1.2 ng/ml, even after induced ovulation, and anovulatory estrus appeared 6 days after this ovulation. In cow H-53, however, the level rose to 2.5 ng/ml 6 days after induced ovulation, when feeble estrus was noticed, and ovulation took place on the following day. In cow S-78, the level ranged from 0.3 to 1.0 ng/ml up to 4 days after the induced ovulation, when silent estrus was noticed.

Figure 3 shows the plasma progesterone level in two cows (J-1 and J-2) of group III after induced ovulation. This level rose rapidly, reaching 5.7 and 8.8 ng/ml in cows J-1 and J-2, respectively, 6 days after induced ovulation. The level was much higher in this group as compared to those of group I or II. It remained high for the following 12 days (5.4 ng/ml on the average) in cow J-2, in which normal luteinization took place. It began to fall rapidly, however, in cow J-1 7 days after induced ovulation, decreasing to almost the same level as determined at the time of ovulation 3 days later. In this cow, the corpus luteum was small in size.

DISCUSSION

Intramuscular injection of PGF$_{2\alpha}$-A (0.8–1.0 mg) and GnRH-A (200 μg) is expected to cause regression of the corpus luteum followed by normal ovulation [7, 16, 17]. Cows of three breeds ranging from 428 to 739 kg in weight were employed in the present experiment. Judging from the results reported by previous workers, the luteal regression and induced ovulation have no relationship to breed and body weight. In groups I and II a dose of 1,000 μg, in addition to 200 μg, of GnRH-A was injected into cows to induce ovulation with assurance. There was little difference in ovulation or in luteinization between the two doses. Therefore, there seems to be no difference between the two doses of the
agent in reactivity of cows to injection with GnRH-A.

Louis et al. [10] and Stellflug et al. [15] intramuscularly injected cows with 30 mg of PGF<sub>2α</sub> in the luteal phase. They found that LH was released 64.0–65.3 hr and ovulation was induced 90–104 hr after the injection. We carried out an experiment on the synchronization of estrous cycle in cows using PGF<sub>2α</sub>-A and found that in most of the cows ovulation was induced 4 days later. In the present experiment, it was found that ovulation was induced earlier in cows injected with PGF<sub>2α</sub>-A and GnRH-A than in those injected with either PGF<sub>2α</sub> or PGF<sub>2α</sub>-A. This tendency was especially obvious in the subjects belonging to groups I and II.

The interval between ovulation after PGF<sub>2α</sub>-A injection and the subsequent estrus was 19.5 days on the average, and the plasma progesterone level became high in all the cows 4–5 days after ovulation. These results indicate the occurrence of normal luteinization. Kaneda et al. [6] induced ovulation followed by the normal luteinization after the injection with GnRH-A which had been preceded by the injection of PGF<sub>2α</sub> longer than 57 hr beforehand. Ovarian follicles were still developing in the cows of groups I and II after injection with PGF<sub>2α</sub>-A when induced ovulation was carried out. Hence, in these cows ovulation was forced to take place very early in the course of development of follicles. This resulted in unsatisfactory luteinization and low plasma progesterone level in every cow, indicating that the occurrence of luteal hypoplasia is closely related to the maturation stage of a follicle at the time of ovulation. When luteinization was observed in the three cows of group III, it was satisfactory in one cow and poor in the others. In an experiment conducted by Kaneda et al. [6] cows were injected in almost the same manner as those of group III with GnRH-A 42–47 hr after injection with PGF<sub>2α</sub>, resulting unsatisfactory luteinization in some. Therefore, the time when the cows of group III were injected with GnRH-A can be regarded as critical as far as luteinization is concerned.

There are only a few investigators who have tried to clarify the cause of luteal hypoplasia. Schams et al. [14] suggested that the hypoplastic corpus luteum that were frequently observed in postpartum cows after the first ovulation following parturition might be related to a fall in plasma LH concentration or a decrease in LH receptors in the corpus luteum.

Lamming et al. [9] studied changes in GTH and progesterone levels over a period from parturition to recurrence of estrus. When the plasma or milk progesterone level rose rapidly within a short period, changes in plasma LH level were not seen as responsible for the rapid rise. The short-lived corpus luteum observed in their experiment was not attributed to hypofunction of the hypophysis, because the hypophysis responded well to synthetic LH-RH. Hare et al. [4], however, induced LH release and ovulation by synthetic LH-RH in seasonally anestrous ewes and observed consistently low levels of plasma progesterone as well as reduced luteal function after ovulation. Furthermore, they [5] studied LH release and progesterone secretion in LH-RH-treated ewes pretreated with PMS, estrogen, or none. The plasma progesterone level was higher in the PMS group than in the control group without pretreatment. There was no difference between the estrogen and the control group. Moreover, there was no relationship between the amount of LH at the time of ovulation and the plasma progesterone level. The authors [5] concluded that in seasonally anestrous ewes, the formation of a corpus luteum, which causes a reduced ability to secrete progesterone, is related to the maturation stage of a follicle at the time of ovulation. Their conclusion supports the present au-
authors' view on the etiology of luteal hypoplasia in cows.

Luteal hypoplasia may be caused by degeneration of corpora lutea, but this aspect was not investigated in the present study. When PGF$_{2\alpha}$ is administered to cows, its degenerative effect on corpora lutea, if any, should be displayed 5 days or later after ovulation. However, an effect was in the present study. Milvae and Hansel [13] administered oxytocin to heifers at 2–6 days of the estrous cycle, and observed an increase in the intrinsic PGF concentration in the uterus and decrease in plasma progesterone level starting at 5 days of the estrous cycle.

In the present experiment, abnormality in luteinization was already noticed in some cows within 5 days after the induced ovulation. A follicle began to develop immediately after induced ovulation and another ovulation took place 4 days after the induced ovulation in some cows. Moreover, plasma progesterone level did not substantially rise after the induced ovulation. The presence of corpus luteum was not confirmed at the site of ovulation in others. These results indirectly suggest that the luteal hypoplasia was not caused by degeneration of corpus luteum, but the evidence lacks definite proof on this point.

The results of the present study support that luteal hypoplasia is produced when ovulation has been forced to take place in the early course of follicle development. The results also suggest a close relationship between the etiology of luteal hypoplasia and the maturation stage of a follicle at the time of ovulation.

ACKNOWLEDGEMENTS. The authors gratefully acknowledge the help of the Takeda Chemical Industries, Ltd. and the Ono Pharmaceutical Industry Co., Ltd. in supplying gonadotropin releasing hormone analogue, TAP-031, and prostaglandin F$_{2\alpha}$ and prostaglandin F$_{2\alpha}$ analogue, ONO-1052, respectively.

REFERENCES


要約

牛における誘起排卵による黄体形成不全：大浪洋二・菊池隆宏・大沼秀男（北里大学獣医畜産学部獣医繁殖学教室）——排卵時の卵胞の成熟度を観察して、牛の黄体形成不全の実験的発生を試みた。黄体期の成牛に対しPGF2α-analogue 1.0 μgを投与後、16時間（Ⅰ群：4頭）、32時間（Ⅱ群：4頭）および48時間（Ⅲ群：3頭）にGnRH-analogue 200または1,000 μgを投与した。Ⅰ群、Ⅱ群およびⅢ群には、それぞれGnRH-analogue注射後平均37.5、20.0および24.7時間に排卵が誘起された。Ⅰ群とⅡ群、計8頭の誘起排卵後の黄体形成はいずれも不良で、性周期は著しく短縮した。Ⅲ群の黄体形成は3頭のうち1頭が良好であったが、他の2頭の黄体は小型であった。Ⅰ群4頭、Ⅱ群3頭、Ⅲ群2頭について誘起排卵後の血中progesteroneの消長を調べたが、Ⅰ群とⅡ群は低値のまま推移し、Ⅲ群は黄体形成の良好な例では正常性周期とほぼ同じ経過をたどり、小型黄体の例では短期間の増加が認められた。以上の成績から、排卵時の卵胞成熟度と黄体形成不全の成因との関連が示唆された。