

## Studies on the Reproductive Phenomena in the Post-partum Rat II. Ovulating Hormone Release in the Post-partum Ovulation in Rat

|                              |   |
|------------------------------|---|
| 著者                           | HASHIZUME Kazuyoshi, SUGAWARA Shichiro, TAKEUCHI Saburo                           |
| journal or publication title | Tohoku journal of agricultural research   |
| volume                       | 24  |
| number                       | 3   |
| page range                   | 123-127   |
| year                         | 1974-02-15  |
| URL                          | <a href="http://hdl.handle.net/10097/29660">http://hdl.handle.net/10097/29660</a> |

## Studies on the Reproductive Phenomena in the Post-partum Rat

### II. Ovulating Hormone Release in the Post-partum Ovulation in Rat

Kazuyoshi HASHIZUME, Shichiro SUGAWARA and Saburo TAKEUCHI

*Laboratory of Animal Reproduction, Faculty of Agriculture,  
Tohoku University, Sendai, Japan*

(Received August 21, 1973)

#### Summary

The timing of OH release in post-partum rats was investigated under two conditions (natural and controlled lighting). In the rats delivered from 11:00 to 20:00 hr on day 22 of pregnancy (day 1 of delivery) under natural conditions, the blocked ratio with sodium pentobarbital at 0, 1, 2, 3 and 4 hours after delivery were 8/11, 4/8, 1/8, 0/1 and 1/2, respectively. On the other hand under controlled light conditions, the blocked ratio with this drug at 0, 1 and 2 hours after delivery were 15/22, 3/11 and 0/6, respectively. Also, in the rats delivered from 8:00 (day 2) to 20:00 hr (day 2), the blocking ratio was almostly similar to that in day 1 of delivery under both conditions. The blocking ratio decreased with the advance of time after delivery. But, in the rats delivered between 20:00 (day 1) and 8:00 hr (day 2), ovulation was blocked very little under either condition. These results indicate that the OH release of each animal in post-partum ovulation is closely related to the end of delivery.

It has been previously reported by this author (1) that the post-partum ovulation in rats occurred between 12 and 30 hours after delivery, and that the time of ovulation was closely related to the end of delivery and the time of delivery. Also, all of the rats which had delivered from 20:00 to 23:00 hr did not ovulate by 30 hours after delivery, and the majority of the rats delivered between 11:00 and 20:00 hr had ovulated by 14 hours after delivery. It may indicated that the ovulating hormone (OH) release takes place from 0 to 18 hours after delivery, assuming 12 hours of latent period from release of OH to ovulation (2-4). Although Hoffman and Schwartz (5) have shown that the OH release responsible for post-partum ovulation in rats appeared to be occurring no earlier than 10:00 hr and no later than 18:00 hr, and their report was agreed with other work (6), the exact relationship between the time of ovulation and that of OH release in rats remains unknown.

This experiment was undertaken to determine the timing of OH release in post-partum rats.

### Materials and Methods

The rats used in this experiment were mature virgin females of the Wistar strain inbred in our laboratory. The rats were kept in a controlled lighting room (12 hrs, light and 12 hrs, dark, light on 6:00 to 18:00 hr) or under natural condition. Vaginal smears were taken daily on at least three consecutive cycles. The rats which had regular 4 day cycles were used. The females in proestrus were caged with fertile males overnight and mating was confirmed by the presence of sperm or plug in vaginal smear on the next morning. This day was designated as day 1 of pregnancy. Parturition was observed every hour from 8:00 hr on day 22 of pregnancy. The determination of the timing of OH release in the post-partum ovulation was performed by the routine method, in which the stimulation in the hypothalamus of the animal was blocked by sodium pentobarbital. Sodium pentobarbital (37.5 mg/kg) was injected subcutaneously at the time calculated by assuming 12 hours latent period for the OH from the previous report (1). All animals were killed from 18 to 24 hours after sodium pentobarbital treatment. The presence of ova in their oviducts was examined for determination of the OH release.

### Results

#### 1) *Natural Condition*

In the rats delivered between 11:00 and 20:00 hr on day 22 of pregnancy (day 1 of delivery), the blocking ratios of ovulation with sodium pentobarbital at 0, 1, 2, 3 and 4 hours after delivery were 8/11, 4/8, 1/8, 0/1 and 1/2, respectively. The blocking ratio seemed to decrease with advancing of time after delivery. But, in animals delivered between 20:00 (day 1) and 8:00 hr (day 2), the ovulation was very seldom blocked. Also, in the rats delivered between 8:00 (day 2) and 17:00 hr (day 2), the ratios of inhibiting ovulation with sodium pentobarbital at 0, 1, 2, 3, 4 and 5 hours after delivery were 5/12, 2/8, 4/8, 2/7, 1/4 and 0/5, respectively (Table 1).

#### 2) *Controlled Lighting Group*

In the rats delivered from 11:00 (day 1) to 20:00 hr (day 1), the ratios of inhibiting ovulation with the drug at 0, 1 and 2 hours after delivery were 15/22, 3/11 and 0/6, respectively. Also, in the rats delivered from 8:00 (day 2) to 20:00 hr (day 2), the blocking ratios of ovulation at 0, 1, 2 and 3 hours after delivery were 7/11, 1/8, 0/1 and 0/5, respectively. The blocking ratio seemed to decrease with the advance of time after delivery. But, in animals delivered between 20:00 (day 1) and 8:00 hr (day 2), the ovulation was blocked in only one case (Table 2).

TABLE 1. *Ratio of Inhibiting Ovulation with Nembutal. Natural*

| Time of Nembutal injection after delivery (hrs) | Time of delivery |     |     |        |     |       |     |     |     |     |        |         |
|---|------------------|-----|-----|--------|-----|-------|-----|-----|-----|-----|--------|---------|
|   | Day 1            |     |     |        |     | Day 2 |     |     |     |     |        |         |
|   | 11-              | 14- | 17- | 20-    | 23- | 2-    | 5-  | 8-  | 11- | 14- |        |         |
| 0   | 2/2              | 4/5 | 2/4 | (8/11) |     |       |     | 0/1 | 2/7 | 3/4 | (5/12) | (13/23) |
| 1   | 1/1              | 1/3 | 2/4 | (4/8)  |     |       |     | 0/1 | 1/4 | 1/3 | (2/8)  | (6/16)  |
| 2   | 0/1              | 0/4 | 1/3 | (1/8)  |     |       |     |     | 3/4 | 1/4 | (4/8)  | (5/16)  |
| 3   | 0/1              |     |     | (0/1)  |     |       |     | 1/3 | 1/3 | 0/1 | (2/7)  | (2/8)   |
| 4   | 1/1              | 0/1 |     | (1/2)  |     |       |     | 1/2 | 0/2 |     | (1/4)  | (2/6)   |
| 5   |                  |     |     |        |     |       | 0/2 |     | 0/3 |     | (0/5)  |         |
| 6   | 0/1              | 0/1 |     | (0/2)  |     |       |     |     |     |     |        |         |
| 7   |                  |     |     |        |     |       |     |     |     |     |        |         |
| 8   |                  |     |     |        |     |       | 0/1 |     |     |     | (0/1)  |         |
| 9   |                  |     |     |        |     |       |     |     |     |     |        |         |
| 10  |                  |     |     | 0/1    | 0/1 | 1/2   |     |     |     |     | (1/4)  |         |
| 11  |                  |     |     |        |     |       |     |     |     |     |        |         |
| 12  |                  |     |     | 0/1    |     | 0/1   |     |     |     |     | (0/2)  |         |
| 13  |                  |     |     | 0/1    |     |       |     |     |     |     | (0/1)  |         |

TABLE 2. *Ratio of Inhibiting Ovulation with Nembutal. Controlled*

| Time of Nembutal injection after delivery (hrs) | Time of delivery |      |     |         |     |       |     |     |     |     |     |        |         |
|---|------------------|------|-----|---------|-----|-------|-----|-----|-----|-----|-----|--------|---------|
|   | Day 1            |      |     |         |     | Day 2 |     |     |     |     |     |        |         |
|   | 11-              | 14-  | 17- | 20-     | 23- | 2-    | 5-  | 8-  | 11- | 14- | 17- |        |         |
| 0   | 1/3              | 8/11 | 6/8 | (15/22) |     |       |     | 1/1 | 3/6 | 3/3 | 0/1 | (7/11) | (22/33) |
| 1   | 0/2              | 1/6  | 2/3 | (3/11)  |     |       |     | 0/1 | 0/4 | 1/3 |     | (1/8)  | (4/19)  |
| 2   | 0/1              | 0/3  | 0/2 | (0/6)   |     |       |     |     | 0/1 |     |     | (0/1)  | (0/7)   |
| 3   |                  |      |     |         |     |       |     | 0/2 | 0/1 | 0/2 |     | (0/5)  |         |
| 4   |                  |      |     |         |     |       | 0/3 | 0/1 |     |     |     | (0/4)  |         |
| 5   |                  |      |     |         |     |       |     |     |     |     |     |        |         |
| 6   |                  |      |     |         |     |       | 0/1 | 0/1 |     |     |     | (0/2)  |         |
| 7   |                  |      |     |         |     |       | 0/1 |     |     |     |     | (0/1)  |         |
| 8   |                  |      |     |         |     | 0/3   |     |     |     |     |     | (0/3)  |         |
| 9   |                  |      |     |         |     | 0/1   | 0/1 | 0/1 |     |     |     | (0/3)  |         |
| 10  |                  |      |     |         |     | 1/2   | 0/2 |     |     |     |     | (1/4)  |         |
| 11  |                  |      |     |         |     |       | 0/1 | 0/1 |     |     |     | (0/2)  |         |
| 12  |                  |      |     |         |     |       |     |     |     |     |     |        |         |
| 13  |                  |      |     | 0/1     |     |       |     |     |     |     |     | (0/1)  |         |
| 14  |                  |      |     | 0/1     |     | 0/1   |     |     |     |     |     | (0/2)  |         |
| 15  |                  |      |     | 0/1     | 0/1 |       |     |     |     |     |     | (0/2)  |         |
| 16  |                  |      |     | 0/2     |     |       |     |     |     |     |     | (0/2)  |         |
| 17  |                  |      |     | 0/1     |     |       |     |     |     |     |     | (0/1)  |         |

**Discussion**

It has been well known that OH release can be blocked by treatment of sodium pentobarbital between 14:00 and 16:00 hr in the cyclic rats (2-4). Also, it has been described by Hoffman and Schwartz (5) that the critical period of post-partum ovulation is longer than that of cyclic ovulation and OH release for post-

TABLE 3. *Ratio of Inhibiting Ovulation with Nembutal. Natural*

| Time of Nembutal injection (hr) | Time of delivery |     |     |     |     |       |     |     |     |            |
|---------------------------------|------------------|-----|-----|-----|-----|-------|-----|-----|-----|------------|
|                                 | Day 1            |     |     |     |     | Day 2 |     |     |     |            |
|                                 | 11-              | 14- | 17- | 20- | 23- | 2-    | 5-  | 8-  | 11- | 14-        |
| 8                               |                  |     |     | 0/2 |     |       |     |     |     | (0/2)      |
| 9                               |                  |     |     |     |     |       |     |     |     |            |
| 10                              |                  |     |     | 0/1 | 0/1 |       |     | 0/1 |     | (0/3)      |
| 11                              | 1/1              |     |     |     |     |       |     | 0/2 |     | (1/3)      |
| 12                              | 1/1              |     |     |     |     |       | 0/2 | 1/2 | 1/4 | (3/9)      |
| 13                              |                  |     |     |     |     | 1/1   |     | 0/1 | 1/6 | (2/8)      |
| 14                              | 1/1              | 1/1 |     |     |     | 0/1   | 0/1 | 1/1 | 2/4 | 1/1 (6/10) |
| 15                              | 0/1              | 1/2 |     |     |     | 0/1   |     |     | 2/3 | 1/3 (4/10) |
| 16                              | 0/1              | 3/5 |     |     |     |       |     |     | 1/3 | 2/4 (6/13) |
| 17                              | 1/1              | 0/2 | 1/3 |     |     |       |     |     | 0/1 | 1/2 (3/9)  |
| 18                              |                  | 0/3 | 1/3 |     |     |       |     |     | 0/2 | 0/2 (1/10) |
| 19                              | 0/1              |     | 2/2 |     |     |       |     |     |     | (2/3)      |
| 20                              |                  |     | 1/2 |     |     |       |     |     |     | (1/2)      |
| 21                              |                  | 0/1 | 0/1 |     |     |       |     |     |     | (0/2)      |

TABLE 4. *Ratio of Inhibiting Ovulation with Nembutal. Controlled*

| Time of Nembutal injection (hr) | Time of delivery |     |     |     |     |       |     |     |     |     |            |
|---------------------------------|------------------|-----|-----|-----|-----|-------|-----|-----|-----|-----|------------|
|                                 | Day 1            |     |     |     |     | Day 2 |     |     |     |     |            |
|                                 | 11-              | 14- | 17- | 20- | 23- | 2-    | 5-  | 8-  | 11- | 14- | 17-        |
| 8                               |                  |     |     |     |     |       |     |     |     |     |            |
| 9                               |                  |     |     | 0/1 |     |       |     | 0/1 |     |     | (0/2)      |
| 10                              |                  |     |     | 0/1 | 0/2 | 0/1   | 0/1 | 1/1 |     |     | (1/6)      |
| 11                              | 0/1              |     |     | 0/1 | 1/1 | 0/2   | 0/3 | 0/1 | 1/3 |     | (2/12)     |
| 12                              | 1/1              |     |     | 0/1 |     | 0/2   |     | 0/1 | 1/2 |     | (2/7)      |
| 13                              | 0/2              |     |     | 0/1 |     |       | 0/1 | 0/1 | 1/3 |     | (1/8)      |
| 14                              | 0/2              | 3/5 |     |     | 0/1 | 0/2   | 0/1 | 0/1 | 0/4 | 1/1 | (4/17)     |
| 15                              |                  | 1/4 |     | 0/1 |     |       |     |     |     | 1/2 | (2/7)      |
| 16                              |                  | 5/5 |     |     |     | 0/1   | 0/1 |     |     | 2/3 | (7/10)     |
| 17                              |                  | 0/4 | 3/4 |     |     |       |     |     |     | 0/1 | 0/1 (3/10) |
| 18                              |                  | 0/2 | 4/5 |     |     |       |     |     |     | 0/1 | (4/8)      |
| 19                              |                  |     | 1/3 |     |     |       |     |     |     |     | (1/3)      |
| 20                              |                  |     | 0/1 |     |     |       |     |     |     |     | (0/1)      |

partum ovulation appeared to be occurring no earlier than 10:00 hr and no later than 18:00 hr. Then, the results rearranged from our data according to conclusion by Hoffman and Schwartz (4, 5) are shown in table 3, 4. In this study, the OH release for post-partum ovulation must have occurred from 10:00 to 20:00 hr under both conditions, assuming 12 hrs latent period for OH. But, there is no significant difference in the blocking ratios between the "critical period" (14:00-16:00 hr) and other times under either condition. However, it has been clarified that OH release of each animals in post-partum ovulation was closely related to the end of delivery. Although the time from the end of delivery to post-partum ovulation was no different between the natural and the controlled groups in the

previous report (1), the OH release period for post-partum ovulation in the natural condition group was broader than that of the controlled lighting group. The reason for this difference in blocking efficiency is yet unknown, though there is a difference in OH release for post-partum ovulation between the natural and controlled condition groups. Also, in animals delivered from 11:00 to 20:00 hr under the controlled condition, the release of OH occurred within 2 hours after delivery, and it may be closely associated to the end of delivery. However, in animals delivered from 20:00 (day 1) to 11:00 hr (day 2), the relationship between the end of delivery and the time of OH release could not be clarified in our experiment. It may be considered from the result that the observation of the time of the end of delivery is imcompleted or the assuming of 12 hrs latent period for OH is not exactly correct.

Recently, Ying *et al* (6) have stated that the sequence of events occurring in the 12 hrs preceding ovulation is similar in both cycle and post-partum rats, and timing of OH release is closely related to the time of delivery. So, our results agreed with theirs (4). Therefore, it can be concluded that the timing of OH release for post-partum ovulation might depend on the end of delivery rather than on the "critical period", and this may suggest that post-partum ovulation is closely related to the mechanism of delivery.

The time of OH release determined by sodium pentobarbital in this study disagreed with the peak time of serum LH measured by radioimmunoassay reported by Ying *et al* (6). But, Norman and Greenwald (7) stated that once the ovary is exposed to the critical level of LH for one-half to one and one-half hours, the processes culminating in ovulation are initiated and continued presence of the hormone is no longer required. It could be suggested that the stimulation in hypothalamic-pituitary does not correspond to the serum LH level, and the highest value of serum LH is unnecessary in post-partum ovulation. Also, it may be considered that assuming 12 hrs latent period for OH caused the mistake in post-partum ovulation. Then, it is necessary to compare radioimmunoassay of serum LH level with biological activity of serum LH.

### References

- 1) Hashizume, K., Sugawara, S. and Takeuchi, S., *Tohoku J. Agr. Res.*, (in press).
- 2) Everett, J.W., "*Sex and Internal Secretions*". Vol. 1. Ed. Young, W.C. 3ed., Williams & Wilkins, Baltimore 1961, Part 8.
- 3) Schwartz, N.B., *Recent Proge. Horm. Res.*, **25**, 1. (1969)
- 4) Schwartz, N.B., "*The Hypothalamus*". Ed. Martini, L., N. Motta and F. Franshini., Academic Press, New York, p 515 (1970)
- 5) Hoffman, J.C., and Schwartz, N.B., *Endocri.*, **76**, 620 (1965)
- 6) Ying, S.Y., Gove, S., Fang V.S. and Greep, R.O., *Endocri.*, **92**, 108 (1973)
- 7) Norman, R.L. and Greenwald, G.S., *Anat. Rec.*, **173**, 95 (1972)