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Introduction

It is well-known that multiple ovulations are easily induced by administering LH to the hens having a number of follicles developed by exogenous FSH. However, in the intact laying-hens, even if LH is administered in a broad range of dosage, it is impossible to induce more than one ovulation, which always occurs from the largest follicle in the ovary.

In the hypophysectomized laying-hens, the sensitivity of the mature follicle to exogenous gonadotropins increases with the lapse of time after hypophysectomy (Rothchild and Fraps[9]).

Opel and Nalbandov[8] reported that the double ovulation was enforced, in the hypophysectomized laying-hens, by a single intravenous injection of mammalian LH given 3 to 15 hours after the operation. To explain these multiple ovulations induced in the hypophysectomized hens, Nalbandov[7] proposed a suggestion that ovulation is normally due to nonsupporting of the mature follicle by gonadotropic hormones (FSH containing) and that this hormone-withdrawal makes the follicles capable of ovulating if they receive the signal (LH) to do so.

The present study was conducted to demonstrate, in the hypophysectomized laying-hens, the inhibitory effect of a single intravenous FSH or PMS injection on the multiple ovulations induced by the exogenous ovulation-inducing hormone (OIH).

Materials and Methods

Single-comb White Leghorn pullets, aged 8 to 10 months, were maintained in individual cages under the standard of 14-hours light-days and with commercial food and water freely available. The egg-layings were hourly recorded from 8 a. m. to 6 p. m. for at least three weeks (prior to and during experiments). Only those hens laying four or more eggs in a clutch, with one day pause, were selected for the experiments. The present study consisted of three experiments.

Experiment I was carried out to investigate the inhibitory effect of a single intravenous injection of PMSG or FSH on the multiple ovulations, induced by an administration of OIH to the hypophysectomized laying-hens. This experiment was divided into Trial 1 and Trial 2.

The removal of the anterior lobe of the pituitary was performed 18 to 20 hours prior to the estimated ovulation-time of C_3, by the transbucal method originated by Hill and

Parkes[3]. The operated hens were maintained in the room at the temperature of 28 ± 2°C until they were sacrificed. These hens were divided into three groups as follows: (1) Hypophysectomized control group; only a single injection of chicken anterior pituitary powder (CAP, 4 mg) or ovine LH (0.4 mg) was given 8 hours after the operation. (2) Experimental group 1; a single injection of PMSG (50 IU or 100 IU) or ovine FSH (0.4 mg) was given 6 hours prior to the OIH injection. (3) Experimental group 2; a single injection of PMSG (50 IU) or FSH (0.4 mg) was given 30 minutes prior to the OIH injection. As control for the hypophysectomized hens, sham-operated hens were used. All hens were sacrificed at approximately 12 hours following the OIH injection, to investigate the induced ovulation-time. The ovulation-time was estimated at autopsy by the status quo and the situation of ovulated ova in the oviduct.

Experiment II was designed to clarify the time of the secondary ovulation in case of the double ovulation induced by LH injection in the hypophysectomized hens.

The hypophysectomized hens were administered with LH (0.4 mg) 8 hours following the operation. These hens were anaethetized with an intravenous injection of phenobarbital sodium (26 mg per Kg body-weight) approximately 6 hours after the injection of LH. Under anaethesia, the left side of the abdominal region was incised, parallel to the pubic bone, and this incision was spread with a caponizing spreader, and the incised section was covered with the gauze wetted with 0.9 % saline solution. Taking off this gauze every 10 minutes, the authors observed the occurrence of ovulations until the secondary ovulation finished.

Experiment III was performed to determine the opportune time of FSH injection to inhibit the multiple ovulations induced by an administration of LH in the hypophysectomized hens. Those hens were divided into seven FSH treatment groups and a control group. They were intravenously injected 8 hours after operation with LH of 0.4 mg. FSH treatment groups were, respectively, injected with FSH of 0.4 mg at various periods (2, 3, 4, 5, 6, 7 and 7.5 hours after the operation). All hens were killed approximately 12 hours after LH injection to confirm the occurrence of ovulations and to weigh the ovulated ova.

FSH-like gonadotropins used in this study were PMSG (serotropin, Teikokuzoki) or ovine NIH-FSH-S9, and the ovulation-inducing hormone was chicken anterior pituitary powder (CAP) or ovine NIH-LH-S18. These hormones were injected into a wing vein.

Results

The experiment I was undertaken to investigate whether an administration of FSH (or PMSG) might inhibit the multiple ovulations induced by OIH injection in the hypophysectomized hens. In the first trial, CAP and PMSG were used as the substitutes for LH and FSH respectively, since CAP exhibits properties similer to those of LH, and PMSG has follicle-stimulating activity.

The results obtained in the first trial are shown at Table 1.

All sham-operated hens injected with CAP at 8 hours after the operation ovulated a single ovum. Whereas in the many hypophysectomized hens (control) with a single injection of CAP, the multiple ovulations involving the ovulation of three ova were induced. In this group, five hens ovulated a single ovum, but it was found that in the two hens among them the largest follicles remaining in the ovary, ruptured easily through the stigma in dissecting process at autopsy. It was assumed that those follicles were on the point of being ovulated.

Table 1. Inhibitory effect of PMSG treatment on the multiple ovulations induced by CAP injection in the hypophysectomized hens.

Groups	PMSG injection time before CAP treatment (PMSG injection time after operation)			No. of hens	No. of ovulations			Weights of ova or follicles (g) (Means ±S.D.)				
	6 hrs. (2 hrs.)	1/2 hrs. (7 1/2 hrs.)	0 hrs. (8 hrs.)		single	double	triple	Normal ovulation	Induced ovulation I_1	Induced ovulation I_2	Induced ovulation I_3	Remaining F_1
Sham-operated controls			CAP, 4 mg	5	5			14.8±0.9	12.9±1.2 (89.4)			10.7±0.9 (72.4)
Hypophysectomized controls			CAP, 4 mg	11	5	5	1	14.9±1.2	13.2±1.4 (88.8)	11.4±2.4* (76.2)	—†	9.0±0.8 (60.7)
Exp. group 1	PMSG, 50 IU		CAP, 4 mg	5	5			14.7±1.3	13.2±0.9 (90.5)			10.5±1.3 (71.6)
	PMSG, 100 IU		CAP 4 mg	7	7			15.8±0.7	13.7±1.2 (86.6)			10.6±1.5 (67.4)
Exp. group 2		PMSG, 50 IU	CAP, 4 mg	4		4		16.2±0.8	13.8±1.3 (84.9)			7.8±2.3 (48.5)

I_1: The primary ovulation, I_2: The secondary ovulation, F_1: The largest follicle remaining in the ovary.

* Mean of three hens

The figures in parentheses indicate the percent of the weights of induced ova to those of ova spontaneously ovulated before hypophysectomy.

† The ovum couldn't be weighed since it was discharged into the abdominal cavity and was broken.

Table 2. Inhibitory effect of FSH treatment on the multiple ovulations induced by LH injection in the hypophysectomized hens.

Groups	FSH injection time before LH treatment (FSH injection time after operation)			No. of hens	No. of ovulations			Weights of ova or follicles (g) (Means ± S. D.)				
	6 hrs. (2 hrs.)	1/2 hrs. (7 1/2 hrs.)	0 hrs. (8 hrs.)		single	double	triple	Normal ovulation	Induced ovulation			Remaining
									I_1	I_2	I_3	F_1
Sham-operated controls			LH, 0.4 mg	11	11			16.0±2.3	14.5±1.8 (90.6)			10.2±1.9 (63.3)
Hypophysectomized controls			LH, 0.4 mg	7		3	4	14.7±1.8	12.7±1.5 (86.3)	12.5* (85.1)	5.8±2.3‡ (39.7)	4.5±2.1 (30.7)
Exp. group 1	FSH, 0.4 mg		LH, 0.4 mg	9	4	5		18.4±2.4	16.9±1.1 (91.8)	12.8±1.9† (69.5)		10.5±1.9 (56.8)
Exp. group 2		FSH, 0.4 mg	LH, 0.4 mg	8		6	2	15.7±1.6	14.3±1.4 (90.8)	13.1* (83.3)	9.4* (60.0)	8.2±2.0 (52.7)

I_1: The primary ovulation, I_2: The secondary ovulation, I_3: The third ovulation, F_1: The largest follicle remaining in the ovary.

* Value of a hen, † Mean of two hens, ‡ Mean of three hens.

The figures in parentheses indicate the percent of the weights of induced ova to those of ova spontaneously ovulated before hypophysectomy.

On the other hand, when PMSG (50 IU or 100IU) was injected 6 hours prior to the injection of CAP, no multiple ovulations occurred in the treated hens (Exp. group 1). However, in the case of being administered with PMSG 30 minutes before CAP injection, all the hens ovulated two ova (Exp. group 2).

In the second trial, ovine FSH and ovine LH were used with the same purpose as in the first trial. The results obtained are presented at Table 2.

All sham-operated hens injected with LH 8 hours after the operation, ovulated a single ovum. This result is similar to that of the sham-operated hens treated with CAP in the first trial. In this trial, all the hypophysectomized hens (control) given only a single injection of LH resulted in the multiple ovulations, and the percent of the hens ovulating thrice at a time was about 57% (4/7). When FSH (0.4 mg) was given 2 hours after hypophysectomy, both the single ovulation and the multiple ovulations occurred respectively (Exp. group 1). The rate of the single ovulation was about 44% (4/9). Frequency of the single ovulation in this trial was low as compared with group 1 in the first trial. But it was shown that FSH had a tendency to disturb the multiple ovulations induced by LH.

In contrast, when FSH was administered 30 minutes prior to the LH injection, the ovulation of two or three ova occurred in all the hens (Exp. group 2). This result coincides with that of group 2 in the first trial. In case of the double ovulation, the secondary ova were scarcely caught by the infundibulum and were discharged into the abdominal cavity. There was no difference in the size between the primary ovulated ova in the double ovulation and the ova in the single ovulation, but they were a little smaller than the normal ovulated ova. Whereas the size of the secondary induced ova was apt to be smaller than that of the induced primary and normal ova.

In a few of hens showing multiple ovulations, the two ova were found in the oviduct, separated in the tract by a distance of several centimeters, forming single yolked eggs.

The interval between the time of LH injection and the time of the primary or secondary ovulation induced by LH was particularly investigated in the experiment II.

As described at Table 3, the primary ovum was enforced to ovulate at the range of 5 3/4 to 7 5/6 hours after LH injection. Secondary ovulation was at the range of 6 1/3 to 8 hours after LH injection.

Table 3. The ovulation-time of the primary and secondary ovulations induced by LH injection in the hypophysectomized hens.

Hen's No.	Induced ovulation-time*			
	I_1		I_2	
	hrs.	min.	hrs.	min.
No. 3	6	05	6	30
No. 11	7	50	8	00
No. 33	6	15	6	20
No. 98	5	45	7	15
No. 104	6	10	6	20
No. 110	6	10	7	40

* The lapse time from LH injection to ovulation

I_1: Primary ovulation

I_2: Secondary ovulation

Table 4. Inhibitory effect of a single injection of FSH at various periods between hypophysectomy and LH injection on the multiple ovulations.

Hours after operation		No. of hens	No. of ovulations		Weights of ova or follicles (g) (Means ± S.D.)			
FSH injection	LH injection		single	double	Normal ovulation	Induced ovulation I_1	Induced ovulation I_2	Remaining F_1
2	8	3	3		15.6±0.7	14.0±0.5 (89.7)		11.4±1.0 (73.1)
3	8	6	6		15.8±1.0	14.7±0.9 (93.0)		14.1±0.9 (89.2)
4	8	7	7		16.0±0.9	15.5±1.4 (96.9)		10.7±2.4 (66.9)
5	8	7	6	1	15.4±1.7	13.0±2.7 (84.4)	—†	11.2±1.9 (72.7)
6	8	6	2	4	15.9±1.9	15.2±1.4 (95.6)	12.5* (78.6)	10.3±0.9 (64.8)
7	8	4		4	14.9±0.6	13.7±1.5 (91.9)	—	8.9±1.4 (59.7)
7 1/2	8	3		3	15.1±0.6	15.1±0.8 (100)	—	8.9±0.8 (58.9)
	8	4		4	14.7±0.4	14.1±0.9 (95.9)	9.7* (66.0)	8.2±1.1 (55.8)

I_1: The primary ovulation, I_2: The secondary ovulation, F_1: The largest follicle remaining in the ovary. * Value of a hen

The figures in parentheses indicate the percent of the weights of induced ova to those of ova spontaneously ovulated before hypohysectomy.

† Those ova couldn't be weighted since those discharged into the abdominal cavity and were broken.

In the experiment III, hypophysectomized hens were respectively given a single intravenous injection of FSH at various periods between hypophysecotmy and LH injection.

The intervening periods from operation to FSH injection to inhibit the multiple ovulations induced by LH in the hypophysectomized hens are shown at Table 4.

When FSH was injected within 5 hours after hypophysectomy, almost all the hens ovulated a single ovum. However, in the case of being injected with FSH at 6 hours after operation, the results of ovulations were divided into the single and the double ovulation, i.e. at this time, four hens of six ovulated twice and the other hens, only single ovum.

On the contrary, FSH injected later than 6 hours after hypophysectomy couldn't inhibit the multiple ovulations. In this experiment, It was found also that the follicles except the primary ones were mostly discharged into the abdominal cavity and were broken down. The weights of the largest follicles remaining in the ovary resulted in the single ovulation were nearly equivalent to those of secondary ovulated ova in the double ovulation (Table 4).

Discussion

In this study, when the hypophysectomized laying-hens were injected with OIH at 8 hours after operation, multiple ovulations involving the ovulation of three ova were induced. This result coincided with the report by Opel and Nalbandov[8]. It is assumed that the

ovulatable sensitivity of the follicles in the ovary shall be enhanced by the removal of anterior pituitary. It was also found that the sensitivity of the secondary follicles in the ovary to exogenous LH or CAP seemed to be related with the size of them, because the small sized follicles were rarely enforced to ovulate in this study (Table 1 & 2).

On the other hand, it was clearly shown that the ovulation of secondary follicles induced by LH injection could be successfully inhibited, provided that FSH was injected within 5 hours after hypophysectomy (Table 4).

It is interesting that the weights of the largest follicles in the ovary of the hypophysectomized hens resulted in the single ovulation by FSH treatment prior to LH injection, were nearly equivalent to those of the secondary ova in the double ovulation induced by LH injection.

It is postulated that those follicles remaining in the ovary were to be probably enforced to ovulate by exogenous LH, unless FSH would be injected at a relatively short lapse of time after hypophysectomy. However, when FSH was administered later than 6 hours after the operation, the inhibitory effect of exogenous FSH to the secondary ovulation was to be found no more, because FSH injection just before LH injection might be too late to inhibit the secondary ovulation. It is assumed that continuous action of FSH may be required to inhibit the ovulation.

There have been got a lot of data about the role of FSH to the ovulation in the hen, though the conclusion is left unclarified. It has been reported by Fraps and Riley[2] that consecutive administrations of FSH caused an interuption of the natural ovulation in the intact laying-hens.

On the contrary, it was suggested that FSH played an important role concerning ovulation in the laying-hens (Ferrando and Nalbandov[1]; Kamiyoshi and Tanaka[5]; Imai and Nalbandov[4]). Kamiyoshi and Tanaka[6] reported that FSH augmented the action of LH to induce ovulation in the hens, i.e. the incidence of premature ovulation increased when FSH was injected, either immediately after or 1 hours after the LH injection.

In our study, it was found that FSH administration within 5 hours following hypophysectomy distrubed, at least, the expected secondary ovulations induced by exogenous LH (injected 8 hours after operation). Although it is difficult to explain adequately why the ovulations of follicles except the primary follicles in the ovary of hypophysectomized hens were inhibited in this interval from operation to FSH injection (within 5 hours), it is assumed that the efficacy of accumulated FSH for this intervening period might weaken that of LH injected at 8 hours after the operation, or that the ratio between FSH and OIH concentrations might be related to this inhibition.

From our study described above, although the relevant mechanism of the action of FSH was left unclarified, this result seemed to partially support the suggestion given by Nalbandov that the ovulation was normally due to withdrawal of FSH in the mature follicles.

Finally, from the fact that the primary ovulation-time induced by exogenous LH administration was not influenced by either hypophysectomy or the intermediate administration of FSH, and that it was almost the same as the ovulation-time in the sham-operated hens, it was considered that the sensitivity of primary follicle to ovulation was to be far superior to any other follicles in the ovary. The ovulation of the primary follicle seemed to be strongly destinated by the action of an unknown factor, comparatively long time before the occurring of its ovulation.

Summary

The inhibitory effects of FSH or PMSG administration on the multiple ovulations induced by exogenous ovulation-inducing hormone (OIH) in the hypophysectomized laying-hens were investigated.

Hypophysectomy was performed 18 to 20 hours prior to the estimated time of ovulation of the third follicle in an ovulatory sequence.

When OIH (chicken anterior pituitary powder or ovine LH) was intravenously injected to the hypophysectomized laying-hens at 8 hours after operation, the multiple ovulations involving the ovulation of three ova occurred in the majority of the treated hens.

On the contrary, when the hypophysectomized laying-hens with OIH injection at 8 hours post-operatively were intravenously injected with PMSG or FSH within 5 hours after operation, most of them resulted in the single ovulation. However, when PMSG or FSH was given 5 to 7.5 hours after hypophysectomy to those hens, almost of those ovulated two or three ova.

From the results described above, it was assumed that FSH administration within 5 hours following hypophysectomy might disturb at least the expected secondary ovulation induced by exogenous LH.

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