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Ovulation in Domestic Hens Treated with Synthetic Mammalian like Luteinizing Hormone – Releasing Hormone (LH-RH)

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SYNTHETIC mammalian like LH-RH was injected i.v. and s.c. into laying hens at different times during the ovulatory cycles. An increase in plasma LH was consistenc y noticed comparable in magnitude but shorter in duration than the natural pre-ovulatory surge of LH. I.V. injection did not induce ovulation, while s.c. injection did. The highly effective dose that induced ovulation was 20 ug LH-RH and the follicle ovulate after 8 hr from injection. The follicle is ovulable at about 11 to 13 hr from proceding oviposition.

The existence of releasing hormones in the hypothalamus has been established. Most of the releasing hormones stimulate the pituitary gland to secrete the corresponding hormones. For instance Luteinizing hormone - releasing hormone (LH-RH) stimulates anterior pituitary to secrete ludeihzing hormone (LH) which induce ovulation (Tanaka, Kamiyoshi and Tagami, 1969 and Opel and Lepore, 1972). Mammalian LH-RH had completely indentified, synthesized and involved interesting studies (Matsuo, Bala, Nair, Arimura and Schally, 1971; Matsuo, Arimura, Nair and Schally, 1971, Schally, Nair, Redding and Arimura, 1971; Redding, Schally, Arimura and Matsuo, 1972 and Reeves, Arimura, Schally, Kraget, Beck and Casey, 1972). Studies concern avian LH-RH were based on discomplete purified materials, small number of observations and with unspecific methods (Clark and Fraps, 1967; Opel and Lepore, 1967, 1972; Jackson and Nalbandov, 1969 a,b,c; Tanaka *et al.*, 1969; Follett, 1970 and Casey, Reeves, Harrison and Peterson, 1971).

Normally, ovulation occurs about 30 min after oviposition (Warren and Scott, 1935; Melek, Morris and Jennings, 1973 and Abdelrazik, 1977). Fraps (1970) showed that mid sequence follicle (C_1) will be ovulated a few before their normal time, but the first follicle of a sequence (C_2) will be ovulable many hours before they would normally ovulate. As mammalian LH-RH is now available, thus it is interesting to examine whether it induces ovulation in domestic fowl and when does the follicle acquire the ability to ovulate in response to exogenous LH-RH.

Material and Methods

White Leghorn hens of a commerial strain aged 455 days were used. They were caged individually, diet and water were available all the time and were kept on a lighting schedule of 14 hr light and 10 hr dark (14 L: 10 D). Oviposition time was recorded automatically to the nearest minute. Individual hens were selected on the basis of regular sequence length and normal oviposition times for each egg within the sequence.

Synthetic mammalian like Luteinizing hormonereleasing hormone (LH-RH) concentrations where prepared to be in 0.2ml saline solution. Injections were given at various time during mid-sequence and at the end of sequence, either before or after laying the terminal egg. For determination the optimum dose, solutions were coded in random order so that when doing the injections and scoring the results it was not known that dose had been administered.

Blood samples were withdrawn into heparinized syrings from the brachial vein over a period of 140 min around injection time. The blood was centrifuged shortly after collection for 20 min at 2,700 r.p.m.and 4°, and plasma stored at - 20° for LH assay. Plasma LH was measured by radioimmuncassay (Follett, Scanes and Cunningham, 1972) with the modifications adopted by Abdelrazik (1977).

Results

Effect on plasma LH levels of LH-RH administration at 15 hr and 2 hr after mid sequence or terminal oviposition respectively.

The results of the administration of 20 ug LH-RH to groups of 8 hens either intra venously (i.v.) or subcutaneously (s.c.) at 15 hr after mid sequence oviposition or at 2 hr after terminal oviposition are presented on Fig. 1 and 2. Plasma LH increased to two fold within 20 min and the high values declined to the base levels at about 50 min after i.v. injections. The base values of LH increased twice within 60 min and returned back to the original *Values after 110 min from s.c. injection. Presence of eggs in the oviduct of untreated hens*

Results of killing hens at about 23 hr after mid-sequence oviposition are shown in Table 1. None of the hens had recently ovulated, but all had a hard-shelled egg in the uterus.

Per-mature mid-sequenes ovulations

Intravenous injection of LH-RH. I.V. injection of 20 ug LH-RH either at 15 hr after oviposition of mid-sequence or at 2 hr after terminal oviposition does not induce premature ovulations (Table 2). Subcutaneous injection of LH-RH. Preliminary experiment showed that ovulation induced by s.c. injection of 20 ug LH-RH. Results of the subcutaneous injection of 20 ug LH-RH at various times during the laying cycle (mid-sequence) of the present work are shown in Table 3 and Fig. 3. S.C. of 20 ug LH-RH is highly effective in causing premature ovulation of mid-sequence follicles when given 15 hr after oviposition. Half of the hens ovulated when injected at 13 hr after oviposition or about 6 hr before the rise in plasma LH normally occurs. It is also seems that when ovulation is induced 3hr prematurely (*i.e.* injections 15 hr after

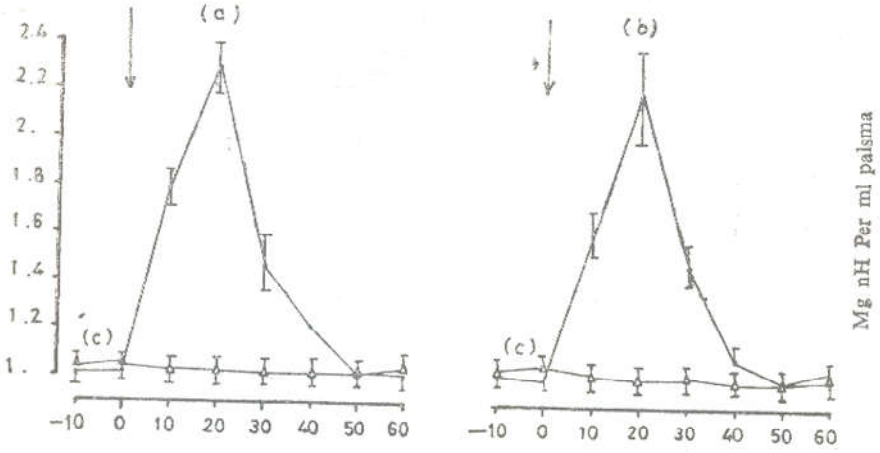

Fig. 1. Pattern of release of luteinizing hormone (mean LH values) in laying hens after I.V. Injection of 20 ug LH releasing hormone (LH—RH) at (a) 15 hr after mid—sequenoe oviposition (b) 2ha after terminal oviposition (c)hens injected with 0,2 ml saline solution. Arrows show time of injection, vertical lines indicate∓ S.E.M.

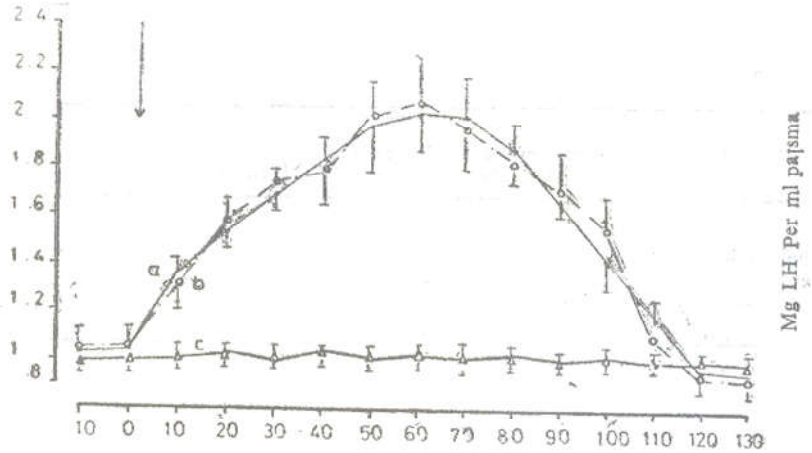

Fig. 2. Effecf of subcutaneous injection of 20 ug Luteinizing hormone releasing hormone (LH —RH) or saline at (a) 15 after mid—sequence oviposition (b) 2hr after terminal oviposition (c) hens injected with 0.2 ml saline solution. Arrow shows tims of injection vertical lines indicate ∓ S.E.M.

TABLE 1. The incidence of eggs in the oviduct of untreated hens mid-sequence).

Hen N°	Time of lay h.m.	Time of killing next day h m.	Egg in uterus
1	9 20	8 10	1
2	10 03	9 00	1
3	8 42	7 50	1
4	9 45	8 38	1
5	10 24	9 12	1
6	8 55	8 00	1
7	9 12	8 06	1
8	9 55	9 05	1
9	8 52	7 44	1
10	10 15	9 11	1

N.B. No eggs were observed in the region of the oviduct

TABLE 2. The effect of intravenous injections of 20 ug synthetic mammalian like LH-RH on ovulation in domestic hens.

Killing time (hours from injection)	Injection at 15 hr after mid-sequence oviposition		Injedction at 2 hr after terminal oviposition	
	No. of hens killed	% of prema-ture ovulation	No. of hens killed	% of—premature ovulation
5	8	—	8	—
6	8	—	8	—
7	8	—	8	—
8	8	—	8	—

TABLE 3. The effect of subcutaneous injection of 20 ug synthetic mammalian like LH-RH at various times during the laying cycle (Mid-Sequence).

Time of inject-ion (hours from oviposition	No. of hens injected	No of hens		
		Ovulated prema-turely and laid	Ovulated prema-turely but did not lay	Not. ovulated
7	10			
9	10	(Not killed)	(half of these birds laid late the next day)	
11	10	0	2	8
13	10	2	3	5
15	10	10	0	0

* These hens had a hard-shelled egg in the shell gland and a yolk in the magnum when examined.

previous oviposition), the next due oviposition is also premature, whereas ovulations induced 5 to 7 hr prematurely do not always cause an associated oviposition.

The interval between subcutaneous injection of 20 ug LH RH and ovulations

Table 4 shows that the highest percentages of ovulations occur after 8 hr from s.c. injection of 20 ug LH-RH. It seems that the median interval from injection to ovulation is about 7 hr.

The optimum dose of LH-RH to induce premature ovulation

Prematurely induced ovulations by various dose of LH-RH are shown in Table 5. The most effective dose induce ovulation is 20 ug LH-RH. 15 ug LH-RH dose induce over 50% ovulation but still fall below the optimum dose.

Premature ovulation at the end of sequence

Table 6 shows the results of injection at the end of a sequence. Subcutaneous injection of 20 ug LH-RH induces ovulation every time when administered in the range from 8 hr before terminal oviposition to 2 hr after terminal oviposition. Ovulations cannot be induced by injections 16 hr before terminal lay. The time so far determined at which C_1 follicle first respond to LH-RH injection is 8 hr before terminal oviposition.

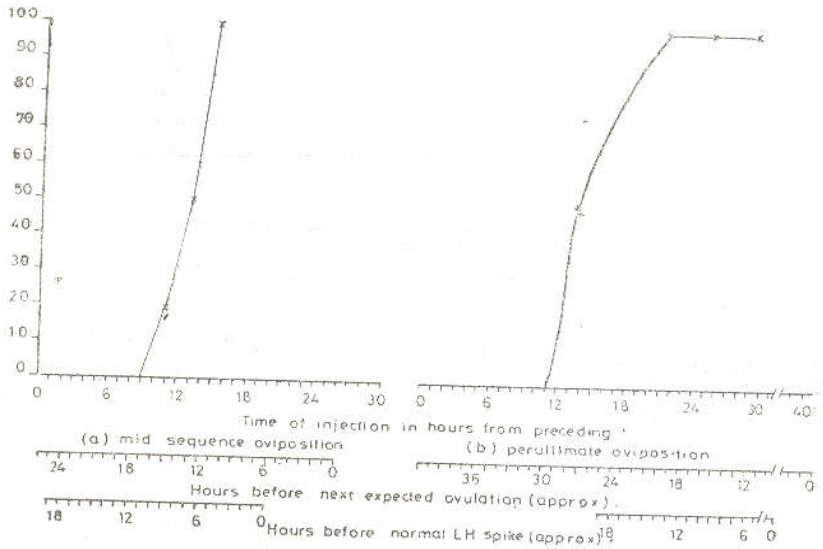

Fig. 3. The relationship between the time of subcutaneous injection of 20 ug LH—RH in 0.2 ml saline and the ability of the folilcle which is destined to ovulate next n respose to injection. Hens killed 8 hr after injection and induced ovulations were distinguished by the presence of an egg in body cavity or upper region of the oviduct.

TALBE 4. The interval from subcutaneous injection of 20 ug synthetic mammalian like LH-RH and ovulation.

Interval (hours) from injection to killing	Injection at 15 hr after mud-sequence oviposition		Injection at 2 hr after terminal oiviposition	
	No. of hens killed	% of induced ovulation	No. of hens killed	% of induced ovulation
5	8	—	8	
6	8	13	8	13
7	8	38	8	50
8	8	87.5		100

TABLE 5. The effect of injecting various concentration of synthetic mammalian-like LH-RH (ug in 0.2 ml saline solution) on ovulation of domestic hens.

Dose ug	No. of hens injected	No. of hens ovulated	% of premature ovulation
20	10	10	100
15	10	7	70
10	10	4	40
00	10	0	0.0

TABLE 6. The effect of subcutaneous injection of 20 ug synthetic mammalian like LH-RH before or after laying the terminal egg.

Time of injection		No. of hens injected	No. ovulated prematurely	No. of premature ovipositions
hours after penultimate oviposition (exact)	Hours around terminal oviposition (approx)			
11	—16	10	—	—
19	— 8	10	4	—
12	— 6	10	10	5
25	— 2	10	10	10
?	+ 2	10	10	10

(—) before terminal oviposition.
(+) after terminal oviposition.

Discussion

The rate of increment of plasma LH value after LH-RH injection was about 100% of the base values which coincide with the natural incremet 4 to 7 hr before ovulation (Cunningham and Furr, 1972; Furr, Bonny, England and Cunningham, 1973; Wilson and Shar, 1973; Senior and Cunningham, 1974; Shedono, Nakamura, Tanabe and Wakabayshi, 1975). Though i.v. injection of the present work did not induce premature ovulation which is in agreement with data reported by Bonney, Cunningham and Furr (1973) and in contrast with data reported by Van Tienhoven and Schally, (1973) who used different materials and secured low percentages of premature ovulation. The duration of LH high values provoked by LH-RH is about 50 min after i.v. injection while the same period after s.c. injedction was longer as found by Cunninhham (personal communication) and shows similarity to the natural peak before ovulation. The pattern of LH peak after injections may explain the failure of i.v. injection and the successful of s.c. injection to induce ovulation.

The premature ovulation after the s.c. injections was due to the effect of LH-RH since examining the hens at about 23hr after mid-sequence oviposition showed one egg only in the uterus without any more in the upper regions of the oviduct. This phenomenon was found by Warren and Scott (1935), Melek *et al.* (1973) and Abdelrazik (1977) who noticed that natural ovulation occurs after oviposition of the hard-shelled egg.

The pecentage of ovulations increased by time till 8 hr after injection when all the injected hens ovulated. This 6 hr interval is normal since the duration of the induced LH peak is about 2 hr and there are 6 hr before the induced ovulation which is nearly equal to the period from natural LH peak to ovulation as reported by (Cunningham and Furr, 1972; Furr *et al.* 1973; Wilson and Sharp, 1973, Senior and Cunningham, 1974 and Etches and Cunningham, 1976).

The subcutaneous injections of LH-RH at either the mid-sequence or around terminal oviposition induce premature ovulation which indicate that the follicle is ovulable at this time. The ovulability of the follicles increased by time which agrees with Fraps (1970) As the follicle is ovulable at about 11 to 13 hr from preceding oviposition, thus follicle is waiting for the permission to LH to be released. It may be said that the ovulable follicle waits for the open period or the signal for LH to be released during the right time (open period).

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تبويض الدجاج عند حقنه بهرمون صناعى مماثل فى تركيبه لهرمون LH - RH الخاص بالثدييات

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حقنت دجاجات بياضة بهرمون مركب صناعى مماثل فى تركيبه لهرمون الثدييات LH-RH الذى ينشط الغدة النخامية لافراز هرمون التبويض .

تم الحقن عند أوقات مختلفة من دورة وضع البيض جمع الدم وقدر الهرمون فى البلازما . وجدت زيادة فى هرمون التبويض بعد الحقن مماثلة لكن لمدة اقصر عن الهرمون الذى يفرز طبيعيا قبل التبويض . أدى الحقن الى التبويض عندما تم تحت الجلد لكن ليس له تأثير عندما تم عن طريق الوريد . وجد أن أمثل كمية من الهرمون الصناعى للحصول على أعلى نسبة تبويض هى ٢٠ ميكروجرام وأن التبويض يتم بعد حوالى ٨ ساعات من الحقن . وجد أن حوصلة الصفار قابل للتبويض عند حوالى ١١ الى ١٣ ساعة من بعد وضع البيض السابقة مباشرة .