HOKKAIDO UNIVERSITY

Title	Notes on Induced Polyovulation in Mature Golden Hamsters (With 12 Text-figures and 2 Tables)
Author(s)	SATO, Akiko
Citation	北海道大學理學部紀要, 15(1), 57-64
Issue Date	1962-12
Doc URL	https://hdl.handle.net/2115/27351
Type	departmental bulletin paper
File Information	15(1)_P57-64.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Notes on Induced Polyovulation in Mature Golden Hamsters

By

Akiko Sato

Zoological Institute, Hokkaido University

(With 12 Text-figures and 2 Tables)

Thanks to the development and improvement of the phase-contrast microscope in recent years, the intimate life processes of gametes and the phenomena of fertilization have been investigated in living eggs, particularly of mammals, without interference from the distortions and artifacts that arise from the use of histological methods. Since 1955, the present author has undertaken studies of experimental polyovulation and superpregnancy in mice and rats following treatment with hormones, together with phase-microscopy observations of those polyovulated eggs (Sato 1956, 1958, 1959a, b, 1962a, b, Ohnuki and Sato 1960). In the present paper, the results of some observations on hormone-induced polyolvulation in hamster eggs are presented as a preliminary report, with special attention to the number of ovulated eggs following the administration of two kinds of hormones.

The author wishes to express her sincere gratitude to Professor Sajiro Makino for his kind direction and improvement of this manuscript. Further cordial thanks are extended to Dr. Eizi Momma and Dr. Yasushi Ohnuki for their keen interest and important advice.

Material and Methods: Golden hamsters (*Mesocricetus auratus*) used as material in this study have been maintained as a strain in the animal house of the Hokkaido University for more than 7 generations. Animals aged approximately 60 days, and all females were mature and virgin.

Female hamsters at various stages of oestrus cycle were injected with 20 i.u. pregnant mares' serum (PMS); they further received an injection of 20 i.u. human chorionic gonadotrophin (HCG), 42, 44, 46, or 48 hours later (at noon on the day of pairing). Two kinds of hormones used were Anteron for PMS and Primogonyl (Schering A.G.) for HCG, respectively. Hamsters received hormones by intramuscular injections. Ovaulation was found to occur during 12 to 16 hours after the injection of HCG. The treated females were placed with males at a rate of 1♀:2♂, and in a period from 6.00 p.m. until 9.00 a.m. the next morning. Then they were examined for the presence of vaginal plugs or spermatozoa in vaginal

Contribution No. 559 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Supported by a grant-in-aid for Fundamental Scientific Research from the Ministry of Education (No. 0438 in 1962).

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 15, 1962.

smear, a sign of successful copulation.

Mated females after hormone treatments were autopsied to observe fertilization at various periods as follows: 10.00 a.m. and 11.00 p.m., on the next day and on the 2nd day after the HCG injection. Fresh eggs were observed by phase-microscopy; for technical details refer to the papers by Sato (1959a, 1962a) and Ohnuki and Sato (1960).

Results

1. *Control data*

Females at the oestrus stage were selected 8.00 to 9.00 a.m. by inspection of their vaginal smear. The animals were mated by being placed with males at the rate of 1♀ : 2♂. The occurrence of mating was detected by the presence of vaginal plugs and spermatozoa in vaginal smear on the following morning.

Mated females were autopsied in order to observe ovulation, fertilization and cleavage at various periods as follows : 10.00 a.m. and 11.00 p.m., on the following day, and on the 2nd day after copulation. Eggs were obtained by the same method as that used for experimental groups, and examined by means of phase-contrast microscopy.

The observations were based on a total of 97 eggs derived from 10 female hamsters. The mean number of eggs ovulated was assessed at 9.7 per individual, with a range of 2 to 16 eggs. Of those 97 eggs, 81 eggs, or 83.5 per cent, were undergoing fertilization, or were at the pronuclear stage and or 2-cell stage of cleavage (Table 1).

Table 1. Number of ovulated eggs following the injection of two kinds of hormones at various intervals.

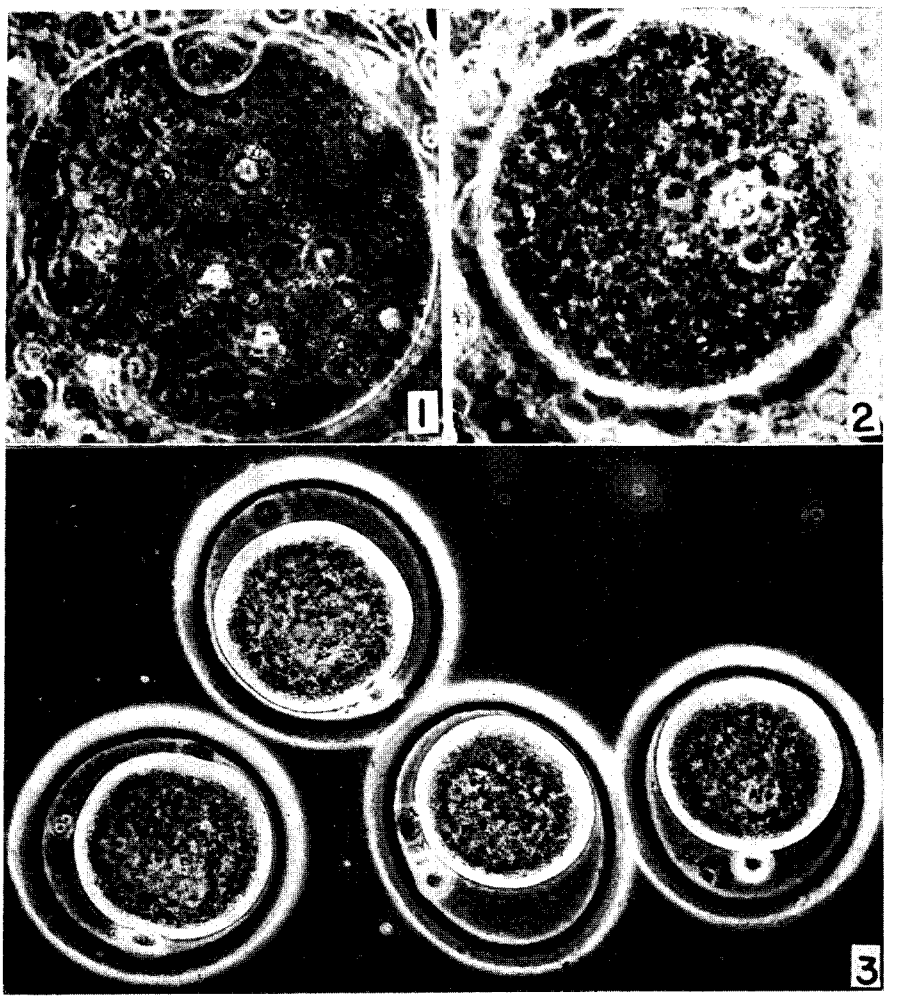
	Interval (hrs)	No. Females	No. Total Eggs	Average No. Eggs
Control	—	10	97	9.7
Experiments	42	6	49	8.2
	44	6	94	15.7
	46	10	263	26.3
	48	6	105	17.5

2. *Experimental data*

The following information was obtained from observations of a total of 511 hormone-induced ovulated eggs taken from 28 treated females.

Experiments were carried out with four different intervals with the use of two kinds of hormones injected. In the first experiment (Experiment I) the interval was 42 hours, in the second (Experiment II) 44 hours, in the third (Experiment III) 46 hours, and in the fourth (Experiment IV) 48 hours respectively.

Experiment I: Six females were treated with a combined application first of 20 i.u. PMS followed with 20 i.u. HCG after an interval of 42 hours. All the females which failed to mate, were killed at 10.00 a.m. on the next day after the HCG injection.



Figs. 1 and 2, an unfertilized egg surrounding the coronal cells. ×300. Fig. 3, early development of eggs; pronuclear stage. ×200.

A total of 49 eggs was obtained from experimental females. The number of ovulated eggs per individual varied from 6 to 10, being 8.2 on an average (Table 1). Microscopical examinations showed that eggs were surrounded by a mass of

cumulus cells and all were unpenetrated. Those eggs carried one polar body and an unusually large perivitelline space (Figs. 1 and 2).

The number of ovulated eggs in this experimental group was smaller than in spontaneous ovulation and in the other three experimental groups.

Experiment II: Six females were treated with the two hormones the second hormone being are interval of 44 hours. All the females which failed to mate, were killed at 10.00a.m. on the next day after the HCG injection.

A total of 94 eggs were obtained from experimental females. The number of ovulated eggs per individual varied from 12 to 19, being 15.7 on an average (Table 1).

The number of ovulated eggs in this group was larger than in Experiment I and in spontaneous ovulation, but smaller than in the other two experimental groups.

Experiment III Ten females which were treated with the two hormones with 46 hours intervening between the respective injections. Eight amongst those 10 females (80.0 per cent) showed vaginal plugs and spermatozoa in vaginal smear as a sign of copulation. Those that had mated were killed at 10.00 a.m. and 11.00 p.m., on the next day and on the 2nd day after the HCG injection; the number of animals from which eggs were recovered was 3, 3, and 2, respectively. The number of eggs extruded in the fallopian tube was 214 in total. The ovulated eggs varied from 18 to 37 in number, being 26.8 on an average per individual (Tables 1 and 2). Among fertilized eggs, 16.0 eggs per individual were found in process of early development, mostly being at the pronuclear stage and at the 2-cell stage of cleavage (Figs. 3–12).

Two females which failed to mate, were killed at 10.00 a.m. on the next day after the HCG injection. The number of eggs extruded in the fallopian tube was 49 in total. Microscopically, eggs were surrounded by a mass of cumulus cells and all were unpenetrated. Those eggs had one polar body and an unusually large perivitelline space.

Table 2. Number of "normal" eggs following the injection of two kinds of hormones at two different intervals.

	Interval (hrs)	No. Females	No. Total Eggs	No. "Normal"	Average "Normal"	Per Cent "Normal"
Control	—	10	97	81	8.1	83.5
Experiments	46	8	214	128	16.0	59.8
	48	4	71	57	14.3	80.3

Note: The term "normal" is used to designate those eggs which appear to be normal in development.

The number of ovulated was larger in this experimental group than in the other experimental groups.

Experiment IV: Six females were injected with 20 i.u. PMS, followed 48 hours later (at noon on the day of pairing) by an injection of 20 i.u. HCG. Out of those

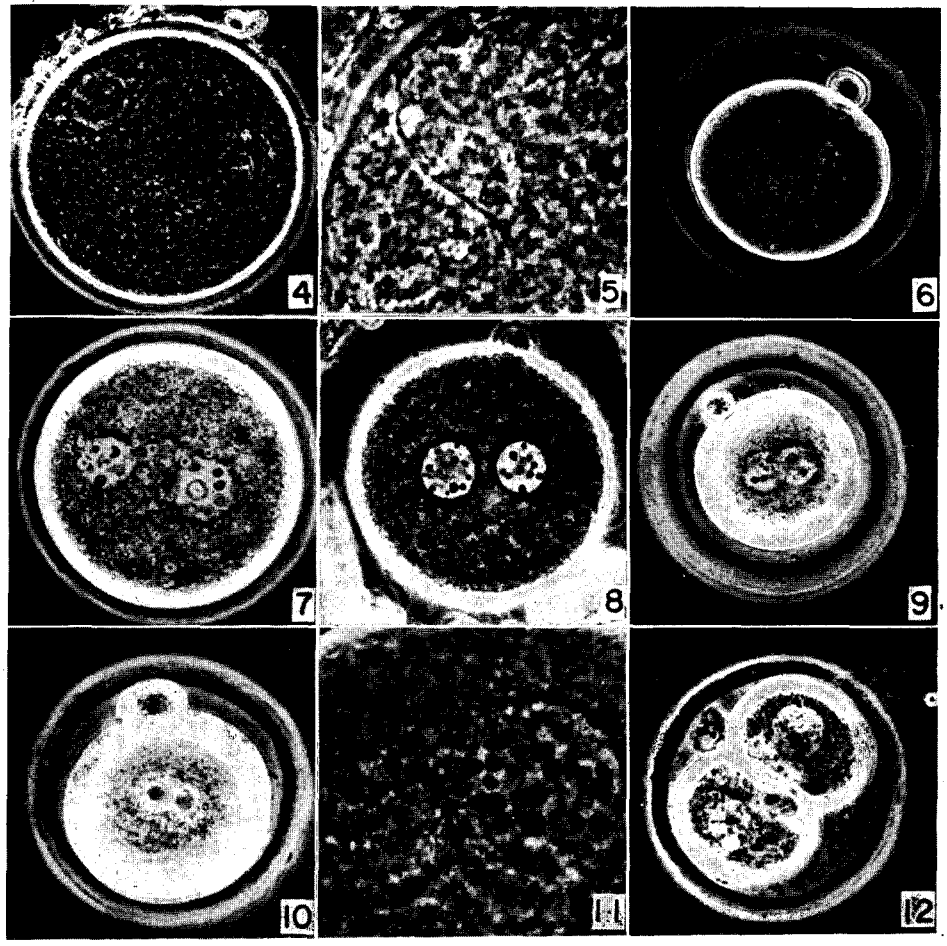


Fig. 4, an ovarian egg with resting nuclei taken from a large follicle. ×300. Fig. 5, a part of the penetrated sperm, showing the tail. ×800. Fig. 6, an egg of early pronuclear stage. ×300. Fig. 7, male and female pronuclei in the process of migration in the egg. ×300. Fig. 8, the same at the later stage. ×300. Fig. 9, an entire eggs in early stage of conjugation of pronculei. ×300. Fig. 10, the same at the later stage. ×300. Fig. 11, two pronuclei in conjugation. ×1000. Fig. 12, an egg at 2-cell stage. ×300.

6 females four mated (66.7 per cent). They were killed at 10.00 a.m., and 11.00 p.m., on the next day after the HCG injection; the animals from which eggs were recovered were 2 and 2, respectively. The number of eggs extruded in the fallopian tube was 71 in total. The ovulated eggs varied from 15 to 20 in number, being 17.8 on an average per individual (Tables 1 and 2). Among fertilized eggs, 14.3 eggs per individual were found in process of early development, mostly being at the pronuclear stage and at the 2-cell stage of cleavage.

Two females which failed to mate, were killed at 10.00 a.m. on the next day after HCG injection. The eggs extruded in the fallopian tube numbered 34 in total, being without insemination.

Discussion

Previous work on fertilization and cleavage in hamster eggs has been made chiefly on fixed material, with a few observations of a general nature having been made on living eggs (Samuel and Hamilton 1942, Graves 1945, Venable 1946, a, b).

The development and improvement in phase-contrast microscopy technique has provided a means whereby the process of fertilization can be studied in living mammalian eggs (Austin and Smiles 1948, Austin 1951, 1956, Strauss 1956, Ohnuki 1959, Yanagimachi and Chang 1961).

Since the pioneer work of Engle (1927), studies on hormone-induced polyovulation and superfecundity in rodents have made striking advances along with the progress of endocrinology (Engle 1927, Pincus 1940, Chang and Marden 1954, Fowler and Edwards 1957, Edwards and Austin 1959).

The present author's studies on induced polyovulation in mice and rats have shown that the number of ovulated eggs after the hormone-treatment may depend on follicle stimulating properties of pregnant mares' serum (PMS) rather than the action of human chorionic gonadotrophin (HCG) (Sato 1962a, b). A combined injection of 40 i.u. PMS and 40 i.u. HCG at 44 hours interval in rats was the most effective to induce the largest number of ovulated eggs in comparison with other doses; 20.6 eggs per individual were obtained in this experiment. In mice a combination of 10 i.u. PMS and 5 i.u. HCG resulted in ovulation of 53.8 eggs per individual. However, Edwards and Austin (1959) succeeded to induce a larger number of eggs in rats by means of a combined treatment of 20 i.u. PMS and 20 i.u. HCG with 48 hours interval between respective injections. It seems to the author that the interval of injection of two kinds of hormones may be an important factor for the induction of ovulation of a comparatively large number of eggs. Further investigation is in progress along this line.

It was found by the author's experiment (Sato 1962c) that 25 r X-irradiation was effective for the implantation of hormone-induced polyovulated eggs in mice.

Summary

Female golden hamsters at various stages of the oestrus cycle were injected

with 20. i.u. PMS; the several groups further received an injection of HCG, 42, 44, 46, or 48 hours later respectively.

In females treated at an interval of 46 hours, the number of ovulated eggs was significantly higher than that in the other three experimental groups. Eight amongst 10 treated females had copulated. In those eight females, the number of eggs extruded in the fallopian tube was 214 in total, varying from 18 to 37 in number, being 26.8 per individual. Microscopically those polyovulated eggs were normal in their general structure. Out of the fertilized eggs, 16.0 eggs per individual were found in process of early development, mostly being at the pronuclear stage and at the 2-cell stage of cleavage.

Literature

Austin, C.R. 1951. The formation, growth, and conjugation of the pronuclei in the rat egg. J. R. micr. Soc., **71**: 295–306.

———— 1956. Ovulation, fertilization and early cleavage in the hamster (*Mesocricetus auratus*). J. R. micr. Soc., **75**: 141–154.

———— and Smiles, J. 1948. Phase-contrast microscopy in the study of fertilization and early development of the rat egg. J. R. micr. Soc., **63**: 13–19.

Chang, M.C. and Marden, W.G.R. 1954. The aerial transport of fertilized mammalian ova. Jour. Hered., **45**: 75–78.

Edwards, R.G. and Austin, C.R. 1959. Induction of oestrus and ovulation in adult rats. J. Endocrin., **18**: 7–8.

Engle, E.T. 1927. Pregnancy following superovluation in the mouse. Proc. Soc. Exp. Biol., N.Y., **25**: 84–85.

Fowler, R.E. and Edwards, R.G. 1957. Induction of superovulation and pregnancy in mature mice by gonadotrophins. J. Endocrin., **15**: 374–384.

Graves, A.P. 1945. Development of the golden hamster, *Cricetus auratus* waterhouse, during the first nine days. Am. J. Anat., **77**: 219—251.

Ohnuki, Y. 1959. A phase microscopy study on the morphological and structural changes in living hamster eggs during ovulation, fertilization and early cleavage. Cytologia, **24**: 348–347.

Ohnuki, Y. and Sato, A. 1960. A cytological study on ovulation, fertilization and early development of artificially polyovulated living eggs of mice. La Kromosomo, **42–43**: 1421–1428.

Pincus, G. 1940. Superovulation in rabbits. Anat. Rec., **77**: 1–8.

Samuel, D.M. and Hamilton W.J. 1942. Living eggs of the golden hamster (*Cricetus auratus*) J. Anat., **76**: 204–208.

Sato, A. 1956. A technique to obtain living mature rat eggs. Yamashina's Inst. Ornithol. Zool., **9**: 373–374.

———— 1959a. Preliminary observations on artificial polyovulation and superfecundity in mature mice. Jap. Jour. Genet., **34**: 226–232.

———— 1959b. Preliminary reports on artificial superfecundity in mature and immature mice. Yamashina's Inst. Ornithol. Zool., **13**: 531–534.

———— 1962a. Some observations on induced polyovulation and superpregnancy in mature mice. Embryologia, **7**: 284-294.

———— 1962b. Induction of superovulation and pregnancy in mature and immature rats

by the application of gonadotrophins. Jap. Jour. Genet., **37**. 253-259.

———— 1962c. On some effects of ionizing radiations upon developing polyovulated mouse eggs. Embryologia, **7**: 384-394.

Strauss, F. 1956. The time and place of fertilization of the golden hamster egg. J. Embryol. Exp. Morph., **4**: 42-56.

Venable, J.H. 1946a. Pre-implantation stages in the golden hamster (*Cricetus auratus*). Anat. Rec., **94**: 105-124.

———— 1946b. Volume changes in the early development of the golden hamster. Anat. Rec., **94**: 129-138.

Yanagimachi, R. and Chang, M.C. 1961. Fertilizable life of golden hamster ova and their morphological changes at the time of losing fertilizability. J. Exp. Zool, **148**: 185-204.