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Author(s)	TSUTSUMI, Yoshio; TAKEDA, Tetsuo; IDA, Motoyasu et al.
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EFFECT OF OXYTOCIN ON THE RECOVERY OF FERTILIZED EGGS FROM THE VAGINAS OF RABBITS

Yoshio TSUTSUMI, Tetsuo TAKEDA, Motoyasu IDA and Satoshi HARA

(Department of Animal Science, Faculty of Agriculture,
Hokkaido University, Sapporo, Japan)

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Introduction

The vaginal washing method for egg recovery from the vaginas of pseudopregnant rabbits was proposed by TSUTSUMI and TAKEDA (1976)[70], although some of unfertilized and degenerated eggs remained in the uterus of pseudopregnant does[2,72]. The nonsurgical recovery of fertilized eggs by this method from estrogen-treated female rabbits[68,71], and from prostaglandin $F_{2\alpha}$-treated does[67] was also made. Some of the eggs recovered from the vaginas were successfully transferred to synchronized recipient does, which eventually developed to newborns.

While oxytocin exerts its functional activity upon the milk ejection and upon the contractile activity of myometrium, especially at the time of parturition, other multiple roles of oxytocin are documented in reproductive physiology. The estrous cycle of cows was shortened by daily administration of oxytocin which was brought about by underdevelopment of the corpus luteum[3,5,9,15,28,29,61,65]. A reduced concentration of progesterone in the luteal tissue was reported by many investigators[11,16,29,35,39,65] with some conflicting results[39,65]. ARMSTRONG and HANSEL (1959)[5] found that oxytocin was ineffective in hysterectomized heifers, but ANDERSON *et al.* (1965)[4] claimed that oxytocin treatment in partially hysterectomized heifers was effective in reducing the interestrous period.

Such effects of oxytocin noted in heifers were not demonstrated in rabbits[10], in rats[10], in hysterectomized rats[38], in hamsters[19] and in the ewe, although some histological degenerative changes were observed in the corpora lutea[40]. However, an unexpected finding was that oxytocin given subcutaneously prior to mating blocked ovulation in rabbits, but the same dose given intravenously at the same time relative to copulation did not show the same blocking effect. The effect of oxytocin was also negligible on

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ovarian follicular development or pituitary FSH activity either in hysterectomized or sham-operated nonsuckled sows which were removed their farrows[45]. According to GINTHER *et al.* (1967)[25] oxytocin produced a shorter estrous cycle in intact heifers and in unicornual heifers in which the retained uterine horn adjacent to the corpus luteum was evident, but not in the unicornual heifers in which the retained horn was in an opposing position to the corpus luteum. This led to the conclusion that part of the effect of oxytocin at least may exert its effect through the local utero-ovarian channels. A diurnal response in corpus luteum function to oxytocin injections was estimated in cows by DONALDSON and TAKKEN (1968)[17]. The early failure of the corpus luteum caused by exogenous oxytocin in cows was dependent neither on the influence of uterine afferent nerve impulses nor on hypothalamic control of the hypophysial function[44], although impaired progesterone synthesis resulted from a decreased ability of treated tissue to convert cholesterol to pregnenolone[77].

SHIBUSAWA *et al.* (1955)[59] suggested that the pituitary-gonadal system is strongly stimulated by an injection of oxytocin in male rats, mongrel dogs and human subjects. In a study on the effects of injections on the course of mammary involution in lactation, BENSON and FOLLEY (1956)[8] stated that release of prolactin, and perhaps other anterior-pituitary hormones related to lactation can be stimulated by treatment with oxytocin. To test the luteotropic properties of various gonadotropins, adoption of the reaction which inhibits the growth of bovine corpus luteum by daily oxytocin injections was proposed by SIMMONS and HANSEL (1962)[60]. Pituitary gonadotropin contents were depleted by about half during estrus or on Day 7 in oxytocin-treated cows[16], and decrease of *in vitro* progesterone synthesis in luteal tissue of heifers treated with oxytocin was not affected by an addition of LH to the incubation medium[11]. It was considered by HARMS and MALVEN (1969)[29] that exogenous progesterone might not prevent depletion of pituitary LH caused by oxytocin. AULETTA *et al.* (1972)[6] proposed an idea that norepinephrine, an alpha adrenergic stimulant, inhibits the luteolytic action of oxytocin in cows.

Recently, ample evidence shows an intimate correlation between oxytocin and prostaglandins. VANE and WILLIAMS (1972, 1973)[75,76] suggested that the uterine response to oxytocin was partially dependent upon the release of prostaglandins in rats and that the increased estrogen production in late pregnancy was accompanied by a marked enhancement of uterine prostaglandin synthesis. Similar results obtained by BAUDOUIN-LEGROS *et al.* (1974)[7] and they thought that estrogen-dependent contractile sensitivity to oxytocin was partially mediated by endogenous prostaglandins in the rat uterus. CHAN (1974)[12)] demonstrated the release of a prostaglandin-like substance into bathed fluid from isolated rat uteri under stimulation of oxytocin.

Although oxytocin had a negligible effect on prostaglandin $F_{2\alpha}$ release in the blood of ewes, the concentration of the prostaglandin $F_{2\alpha}$ rose dramatically within 5 minutes when oxytocin was given to estrogen primed ewes[58)]. Oxytocin, however, caused 4.9-5.3 fold increases in prostaglandin F concentrations in uterine venous effluent in pregnant ewes[41)]. In supporting the view that prostaglandin-synthetic mechanism in the ovine uterus can be extremely sensitive to oxytocin, ROBERTS *et al.* (1975)[50)] argued against, not only the hypothesis that prostaglandins mediate the oxytocin effect on uterine motility, but also against it in a converse manner. High-affinity binding sites of oxytocin were found in both myometrium and endometrium in the ewe, but it was found that oxytocin enhanced release of prostaglandin $F_{2\alpha}$ from endometrial tissue only during incubation. It was suggested that the endometrium is a target for oxytocin, and that synthesis of prostaglandin $F_{2\alpha}$ by the uterus may involve an interaction between oxytocin and its endometrial receptors, and that ovarian steroids may influence uterine prostaglandin synthesis by regulating the availability of these receptors[52)]. Again, ROBERTS and MCCRACKEN (1976)[51)] argued against not only the hypothesis that uterine motility must increase before prostaglandins are generated in response to oxytocin and also against it in converse, *viz.*, that prostaglandins mediate the oxytocic action of oxytocin. According to NEWCOMB *et al.* (1977)[42)] an increase of prostaglandin F in the blood of heifers in response to oxytocin was significantly influenced by the day of the estrous cycle, with the greatest response occurring on Day 3 after estrus. Conversly, GILLESPIE *et al.* (1972)[24)] showed that oxytocin appeared in blood plasma of women during the infusion of prostaglandin E_2 or $F_{2\alpha}$.

Numerous investigations have accumulated regarding the effects of physical stimulations on the reproductive organs. Natural mating or massage of the cervix and cervical os *per rectum*, or insertion of an inseminating tube into the cervix resulted in an increase of intramammary pressure in the cow[30)]. Increased uterine activity occurred during natural mating, and by intravenous injection of oxytocin, similar to that obtained by cervical massage[73)]. The presence of the bull, nuzzling by the bull, noncopulatory mounting, and copulation served as stimuli which resulted in an increased uterine tone and uterine contractions within a few seconds[74)], and these were considered to be derived by the release of oxytocin[31)]. Similar status was also reported in ewes[36)]. Artificial distension of the vagina stimulated release of oxytocin in goats[23,46,47,49,53,54], and in ewes[21,53]. The vaginal distension in late pregnancy or before parturition raised oxytocin levels in blood plasma of ewes prior to elevation of prostaglandin F levels, and these were considered to support the suggestion that the elevated prostaglandin F levels resulting from vaginal distension are caused by the reflex secretion of oxytocin[21]. According to ROWSON *et al.* (1972)[57], however, no detectable amounts of oxytocin were released into the bloodstream of the cow following vaginal and cervical stimulation during the early luteal phase.

Sensitivity of the uterus to oxytocin reached a maximum at proestrus and estrus in rats, and a thirteenfold increase of the sensitivity was attained by three daily injections of stilbestrol[22]. The amount of oxytocin in blood of goats, released in response to vaginal distention, was found to vary with the time of year and this was considered to be related to the seasonal periodicity of estrous behavior and ovulatory activity[47]. Estradiol stimulated uterine contractions and increased both the sensitivity and intra-uterine pressure response to oxytocin in the goat[33], and the uterine sensitivity was greater in the estrous phase[34]. Vaginal stimulation induced greater elevations in the level of oxytocin in caprine blood near the time of estrus and during the early luteal phase as compared with periods characterized by maximal concentrations of progesterone[49]. These facts suggested that fluctuations in endogenous ovarian hormones during the estrous cycle may influence the oxytocin secretory mechanism or the sensitivity of uterus to oxytocin. In fact, infusion of progesterone reduced the amount of oxytocin released in response to vaginal distention stimuli in goats, and infusion of estradiol enhanced the oxytocin-releasing response to vaginal distention stimuli in ovariectomized goats[48]. It was recently reported by SOLOFF (1975 a)[62] that enhanced sensitivity of the rat uterus to oxytocin following estrogen treatment was the result of an increase in the affinity and number of oxytocin receptors in the uterus.

Significantly earlier ovulation was reported in the first *post-partum* ewes injected with oxytocin ten times each day for the first 17 days after parturition[20]. Oxytocin triggered a distinct contractile activity of isolated ovaries of rats, and this activity was clearly more marked immediately prior to ovulation. In contrast, the contractions induced by prostaglandin $F_{2\alpha}$ were similar to that in oxytocin dosage during early proestrus and late proestrus[66]. Regardless of the above, no significant differences in the sensitivity of the ovaries of rats to oxytocin in the different phases of the estrous cycle were reported by ROCA *et al.* (1976)[55].

A few of findings are available on the response of the oviduct to oxytocin.

DONALDSON (1969)[15] reported that oxytocin produced epithelial cysts in the fimbria and ampulla of the oviduct and in the endometrial mucosa of cows. Oxytocin stimulated an increase in the amplitude and frequency of contraction of isolated human oviducts[56], and such oxytocin-stimulation to tubal motility was evident at the time of menstruation and during the early proliferative phase of the menstrual cycle[14]. Strips from the isthmic part of the oviducts of ewes showed an increased contractile activity in the presence of prostaglandin E_1 or oxytocin, while the spontaneous activity of the isthmus was unchanged after addition of prostaglandin $F_{2\alpha}$ to the organ bath[18]. When [^{3}H] oxytocin was incubated *in vitro* with pieces of oviduct in the rat, radioactivity was localized in the smooth muscle cells of the oviduct, suggesting the presence of specific and high affinity receptors for oxytocin in the oviduct[64]. Particulate fractions obtained from rat oviductal homogenates had a high affinity to oxytocin, and treatment with diethylstilbestrol for 2 days increased the affinity of oviductal binding sites for oxytocin by 4 fold[63].

These recent communications, in which the roles of oxytocin in relation to the reproductive physiology were investigated, strongly suggested an examination of the effect of oxytocin on the egg transport through the reproductive tract of rabbits, in spite of statement of MAIA *et al.* (1976)[37]. They reported that oxytocin does not have any effect on the rabbit oviduct, while it markedly increases the contractility of the guinea pig and hamster oviducts *in vitro*. It is believed that, if premature entry of eggs into the uterus occurs, the eggs are quickly transported through the uterus and expelled into the vagina[1]. Our results in previous papers[67,68,71] supported this concept, and it was considered that some possibility to recover eggs from the vagina may exist, if acceleration of egg transport occurs by treatment with oxytocin.

Materials and Methods

Forty five Japanese White adult female rabbits received injections of 20 to 40 IU of human chorionic gonadotropin to ensure ovulation after double mating with fertile males. The forty two females of these were divided into six groups according to the dose and time of injection of oxytocin (Showa Yakuhin Kako Co.) and estradiol benzoate (Progynon B oleosum, Schering) (Fig. 1). Group I and II were used for egg recovery by treatment of oxytocin, and Groups III to V were for the recovery by combined treatment of estradiol benzoate and oxytocin. Single treatment of estradiol benzoate was made in Group VI. Does in Group I were given subcutaneous injections of oxytocin in sequence to recover the tubal and uterine eggs as

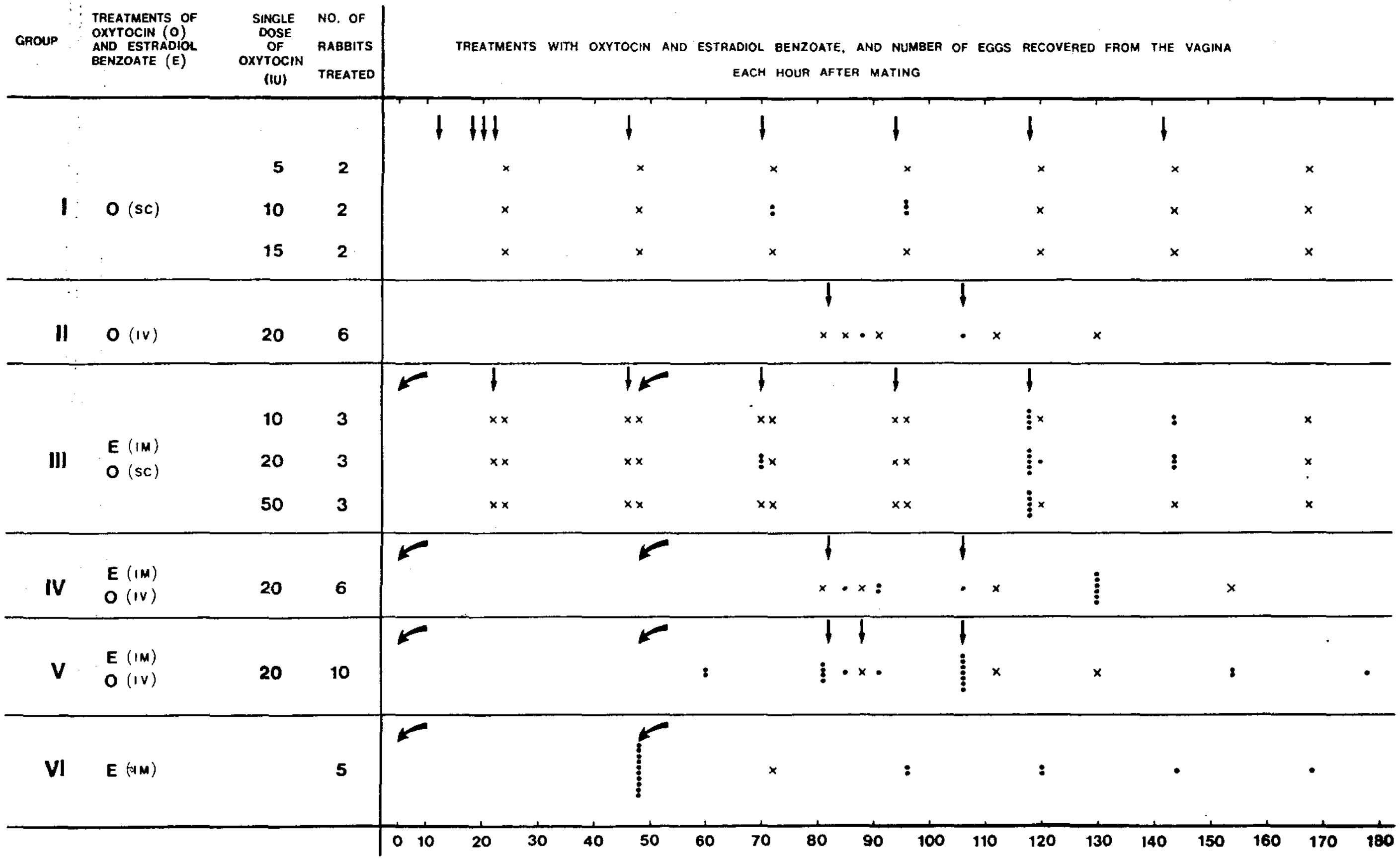

Fig. 1. Treatments with oxytocin and estradiol benzoate, and the recovery of eggs from the vagina. (SC), subcutaneous injection; (IV), intravenous injection; (IM), intramuscular injection; ↙, injection of 10 μg estradiol benzoate; ↓, oxytocin injection; ●, egg recovered; ×, no egg recovered.

TABLE 1. Number of eggs recovered by vaginal washing each hour after mating

Hours after mating	Single treatment of oxytocin (12 does)		Combined treatment of estradiol benzoate and oxytocin (25 does)			Single treatment of estradiol benzoate (5 does)	
	Unfertilized	Cleaved	Unfertilized	Cleaved and/or degenerated	Empty zona pellucida	Cleaved and/or degenerated	Empty zona pellucida
48	•	•	•	•	•	9 (a)	1
60	•	•	•	2 (b)	•	•	•
70	•	•	•	3 (c)	•	•	•
72	2	•	•	•	•	•	•
81	•	•	•	4 (a, d)	•	•	•
85	•	•	•	2 (c, d)	•	•	•
88	•	1 (d)	•	•	•	•	•
91	•	•	•	3 (d)	•	•	•
96	1	2 (d)	•	•	•	2 (d)	•
106	•	1 (d)	•	8 (d)	•	•	•
118	•	•	•	11 (d)	3	•	•
120	•	•	•	1 (d)	•	2 (d)	•
130	•	•	•	6 (d)	•	•	•
144	•	•	•	5 (d)	•	•	1
154	•	•	•	1 (d)	1	•	•
168	•	•	•	1 (d)	•	•	1
Total	3	4	0	47	4	13	3
	7		51			16	

a. 4-, 8-, 16-cell stage b. 16-cell stage c. morula-stage d. blastocyst-stage a pilot examination. Group II was dealt with for recovery of uterine eggs by intravenous injections of oxytocin. All does of Group III to VI received intramuscular injections of 10 μg of estradiol benzoate twice, at the time of mating and at 48 hours after mating. In Group III oxytocin was injected subcutaneously to recover tubal and uterine eggs, and in Group IV and V oxytocin was given intravenously to recover the uterine eggs.

In vivo vaginal washing was made in all does from each hour to 7 days *post coitum* (*p. c.*) as shown in Figure 1. The apparatus for vaginal washing and the washing method were the same reported previously[67,68,70,71]. Some eggs recovered from does in Group II, III and V were transferred to 3 synchronous pseudopregnant recipient does. One doe in Group IV and 6 in Group V were autopsied to locate eggs in the oviduct and uterus at the end of vaginal washing at 178 hours *p. c.*

Location of the eggs in oviducts and uteri of 3 does was identified using the freezing-clearing technique after the method of ORSINI (1962)[43] and HOWE (1970)[32]. These does received 5, 10, or 15 IU of oxytocin at 12, 18, 20 and 22 hours *p. c.* before autopsy at 24 hours *p. c.*, respectively.

Results

1. Number of eggs recovered by the vaginal washing

Seventy four eggs in total were collected from forty two does by vaginal washing on Day 1–7 *p. c.* (Fig. 1). The number and developmental stages of these eggs are shown in Table 1.

In Group I (6 does), 2 unfertilized eggs and one blastocyst were recovered from one doe 72 and 96 hours *p. c.* respectively, and 2 blastocysts were obtained from another doe 96 hours *p. c.*, although multiple injections of oxytocin in sequence were made after 12 hours *p. c.* In Group II (6 does), only 2 blastocysts were found from 2 does 88 and 106 hours *p. c.* after a dose of oxytocin 82 hours *p. c.* While, the rate of egg recovery was markedly elevated by the combined treatment of estradiol benzoate and oxytocin (Groups III to VI). In Group III (9 does) 3 morulae were recovered from a doe 70 hours *p. c.* and 11 degenerated blastocysts from 6 does were recovered 118 hours *p. c.* in concentrated fashion. The vaginal washing was started from 81 hours *p. c.* following the first treatment of oxytocin 82 hours *p. c.* in Group IV (6 does), 3 blastocysts from 3 does 85 to 91 hours *p. c.* and 6 degenerated blastocysts from 3 does 130 hours *p. c.* were recovered. In Group V (10 does), one eight-cell stage egg and 2 blastocysts appeared in 3 does before treatment with oxytocin, and 9 blastocysts were recovered from 5 does 85 to 106 hours *p. c.* Effects of single treatment of estradiol benzoate was examined in Group VI. Ten eggs were obtained from a doe 48 hours *p. c.* and 6 blastocysts were obtained from other does dispersedly.

In general, most of the blastocysts recovered after 106 hours *p. c.* were degenerated microscopically.

TABLE 2. Results of washings of the oviduct and uterus of rabbits treated with a combination of oxytocin and estradiol benzoate and the number of consecutive vaginal washings received before autopsy on 178 hours after mating

Rabbit no.	No. of corpora lutea	No. of eggs recovered by vaginal washings	No. of implantation sites	No. of eggs recovered from oviduct	No. of eggs recovered from uterus	No. of eggs missed
5048	9	0	0	0	3	6
5019	9	2	0	0	0	7
5049	9	0	0	4	3	2
5051	14	0	0	0	1	13
5042	11	3	0	0	0	8
5059	7	1	0	2	0	4
5063	10	5	0	0	2	3
Total	69	11	0	6	9	43

TABLE 3. Effects of oxytocin and estradiol benzoate treatments on the reproductive efficiency in rabbits

Treatment	Single dose of oxytocin (IU)	No. of rabbits treated	No. of rabbits recovered eggs	No. of pregnant rabbits	Total no. of fetuses and newborns	Percentage of pregnant rabbits
Oxytocin	5	2	0	2	9	75.0
	10	2	2	1	1	
	15	2	0	2	10	
	20	6	2	4	35	
Oxytocin and estradiol benzoate	10	3	2	0	0	4.0
	20	3	3	0	0	
	50	3	2	0	0	
	20	6	4	1	2	
	20	10	7	0	0	
Estradiol benzoate	0	5	5	0	0	0.0

2. The reproductive efficiency of oxytocin- and estradiol benzoate-treated rabbits

Seven does in Groups IV and V were slaughtered at the end of the last vaginal washing 178 hours *p. c.* and the eggs in oviducts and uteri were located. The results of the observations are given in Table 2. No implantation sites were observed in the uterus and 9 degenerated blastocysts of various degrees were found in the fluid of uterine washing. Four and two markedly degenerated eggs were still lodged in the oviducts of 2 does, respectively. Table 3 shows the reproductive efficiency in the group of the single treatment of oxytocin, and that of the combined treatment of estradiol benzoate and oxytocin, and also that of the single treatment of estradiol benzoate. It was noted from the results that the treatment in the latter two groups may be toxic for embryo survival.

3. Results of egg transfer

Nine selected eggs recovered from the vaginas of single oxytocin-treated does and from does with combined treatment 70 to 106 hours *p. c.* were transferred to the oviduct or uteri of three synchronous pseudopregnant does in order to test the viability of the eggs (Table 4). No implantation sites, however, were recognized at laparotomy 9 days p. c.

TABLE 4. Results of transfer of eggs recovered from vaginas of oxytocin- and estradiol benzoate-treated rabbits

Treatment of donor	Time of transfer (hr after mating)	No. of eggs transferred (cell-stage)	No. of recipients	Location of egg transfer	No. of implantation sites at 9 days after mating
Oxytocin	88 106	1 (blastocyst) 1 (blastocyst)	1	uterus	0
Oxytocin and estradiol bedzoate	70	3 (morula)	1	oviduct	0
	91 106	1 (blastocyst) 3 (blastocyst)	1	uterus	0

4. Location of eggs by the freezing-clearing technique

Fifteen eggs were detected in the oviducts and no eggs were located in the uteri of three does 24 hours *p. c.* The total number of corpora lutea was 28, and the detectability of eggs was 53.5%. When the positions of eggs in the oviduct were illustrated by the percentage of distance travelled from the ostium of the oviduct, the mean distance travelled by eggs was 51% (range 29-75) of oviductal length and the mean percentage length of ampulla in the oviduct was 47% in this case.

Discussion

It was clearly demonstrated by many investigators, that egg passage through the oviduct was partly regulated by a delicate balance of levels of estrogen and gestagen. Since shortening of the estrous cycle in the cow by administration of oxytocin was reported, the multiple roles of oxytocin on the reproductive physiology were carefully scrutinized as quoted in the present survey. The consensus of the reports is that the effects of oxytocin are related to days in estrous cycle, to the existence of uterine tissue, and to the levels or synthesises of sex hormones and gonadotropic hormones. Further evidences also suggested a intimate connection between oxytocin and prostaglandins synthesis. Thus, it was considered by the present authors that there is a possibility that the rate of egg transport through the reproductive tract may be changed by administrations of oxytocin. If some change occurs in the rate of egg transport, eggs may be expelled into the vagina through the uterine lumen.

Neverthless, the present results seem to show the inability of oxytocin to alter the rate of egg transport through the reproductive tract. Only seven eggs were recovered from 12 does with a single treatment of oxytocin, 51 eggs including 4 empty zonae pellucidae were obtained from 25 does with the combined treatment of estradiol benzoate and oxytocin, and 16 eggs including 3 empty zonae pellucidae were recovered from 5 does with a single treatment of estradiol benzoate. The average number of eggs recovered per doe was 0.58 for the first treatment, and was 2.04 for the second treatment, and 2.23 for the third treatment. Apparently both the combined treatment and the single treatment of estradiol benzoate were more effective than the single treatment of oxytocin. In the previous data of TSUTSUMI *et al.* (1976)[71] 72 eggs were recovered from the vaginas of 32 does treated with estrogen in a similar experimental design. The average number of eggs per doe was 2.25, and this figure is almost similar to that in the Groups III to VI. Furthermore, our unpublished data in preliminary experiments showed evidence that a few eggs are expelled into the vagina in some normal female rabbits during the preimplantation stage. From these facts, it may be stated that the effects of oxytocin appear to be very ineffective or negligible on the recovery of eggs from the vagina.

In general, the time required for egg passage through the oviduct was about 72 hours in normal rabbits. Treatment of oxytocin before 72 hours *p. c.* were for the recovery of the tubal eggs in Groups I and III, and the results of these groups showed that oviducts showed absolutely no response to the treatment of oxytocin. The aim of Group II was to recover the uterine eggs, but only 2 eggs was obtained from 6 does. To recover the uterine eggs oxytocin was administered at or after 82 hours *p. c.* in Groups IV and V, also. And blastocysts appeared in a concentrated fashion 106 to 144 hours *p. c.* No difference, however, was noted in comparison with the previous data of estradiol benzoate treatment71) in the pattern of recovery of uterine eggs. This was also confirmed additionally by the observations of cleared oviducts and uteri. The position and distribution pattern of eggs in the oviduct 24 hours *p. c.* in the present cleared specimens was similar to that in normal rabbits as demonstrated by TSUTSUMI and HAFEZ (1974)69).

It is acceptable that administration of estrogen immediately after ovulation can prevent pregnancy by the disturbance of egg transport and endometrial development, although such effects are dependent on the type of compound, dosage, species and time of administration13). According to GREENWALD (1957)26), when rabbits received 5 μg dose of estradiol benzoate for three consecutive days beginning on 2, 3 and 4 days *p. c.*, the does showed reduced litters, and a 10 μg dose terminated pregnancy. He (1959)27) also reported that 5 μg of estradiol benzoate injected immediately after mating and 48 hours later, led to a loss of 82 per cent of the embryo, and that this was due to the acceleration of the tubal passage of ova and the increase of uterine motility, resulting in expulsion of most of the ova from the uterus at 70 hours after mating. Thus, it is reasonable to expect no pregnancy in the does treated with estradiol benzoate, except in the case of a doe with 2 newborns. The results of egg transfer were negative in the present study, although it may be rash to make a conclusion at this stage. And average litter size (4.6) in the oxytocin group was very small in comparison with the usual size. Neverthless, the possibility as to whether the dose of oxytocin may be effective to embryo survival remains unsolved.

Many investigators reported that physical stimulation of the reproductive tract causes the release of oxytocin which contributes to uterine contractile activity. It has always been considered that there is some doubt regarding the release of oxytocin due to reflective mechanisms to the vaginal washing used to recover eggs, and that such a reflective mechanisms may affect the embryo passage through the reproductive tract. However, the results of the present study showed that such consideration is not necessary concerning the vaginal washing method used, as in the statement of ROWSON *et al.*

(1972)(57) that no detectable amounts of oxytocin are released into the blood stream following stimulation of the vagina and cervix by the introduction of a glass inseminating pipette during the early luteal phase in the cow.

Summary

The multiple role of oxytocin on the reproductive physiology, except for the role on milk ejection and on the contractile activity of myometrium, were surveyed. From the survey an assumption arose in which it was considered that the eggs may be expelled into vagina if some changes occur in the rate of egg transport by the administration of oxytocin which disturb the delicate balance of sex hormones and/or prostaglandins synthesis. The present study dealt with the effect of oxytocin on the recovery of eggs from the vagina.

Forty two female rabbits mated were divided into six groups according to dose and time of dosage of oxytocin and estradiol benzoate. These females were subjected to vaginal washings to recover eggs 1 to 7 days after mating.

The results seemed to show the inability of oxytocin to alter the rate of egg transport and recovery of eggs from the vagina. This was supported by observations of cleared oviducts of 3 does receiving oxytocin, because the position and distribution pattern of eggs in the oviducts were similar to that in normal untreated does. Treatment of estradiol benzoate was effective in the recovery of eggs regardless of treatment with oxytocin. The effect of oxytocin on the embryo survival was not determined. However, it was considered that the vaginal washing method to recover eggs may have no detrimental effects with special regard to egg recovery from the vagina irregardless of the physical stimulation to the vagina.

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Plate 1

Explanation of figures

Fig. 1. A degenerating egg recovered 48 hours *post coitum.* (Group VI). ×174.

Fig. 2. A degenerating egg in 16-cell stage recovered 48 hours *post coitum.* Part of the zona pellucida had burst and opened, and irregular blastomeres in size were observed. (Group VI). ×174.

Fig. 3. A normal egg in 16-cell stage recovered 48 hours *post coitum.* (Group VI). ×174.

Fig. 4. A degenerating egg in 8-cell stage recovered 81 hours *post coitum.* (Group V). ×174.

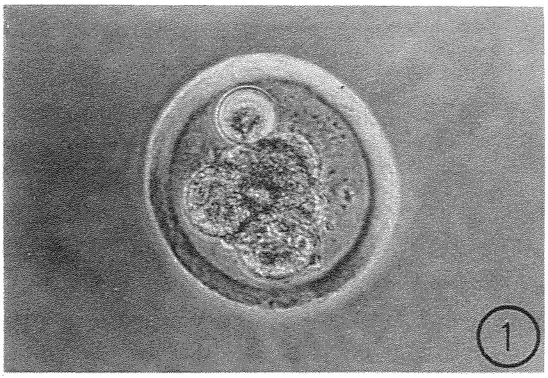
Fig. 5. A normal blastocyst recovered 81 hours *post coitum.* (Group V). ×174.

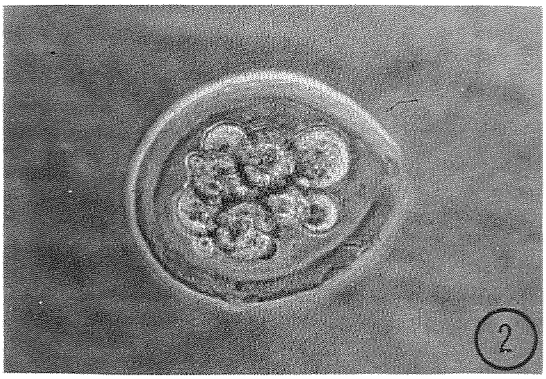
Fig. 6. A normal blastocyst recovered 85 hours *post coitum.* (Group V). ×174.

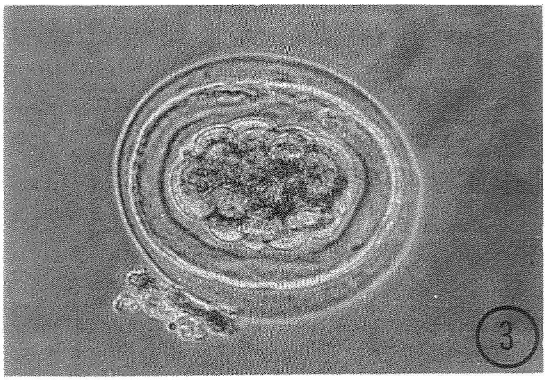
Fig. 7. A normal blastocyst recovered 96 hours *post coitum.* (Group I). ×77.

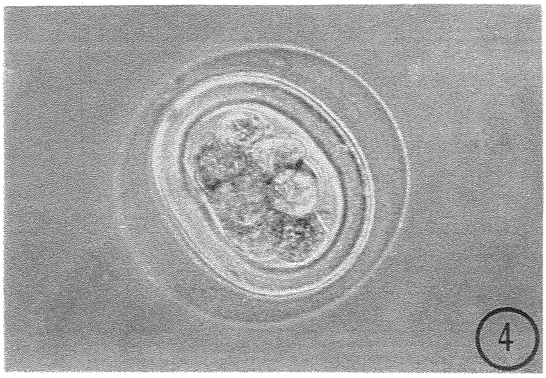
Fig. 8. A degenerating egg recovered 106 hours *post coitum.* (Group V). ×174.

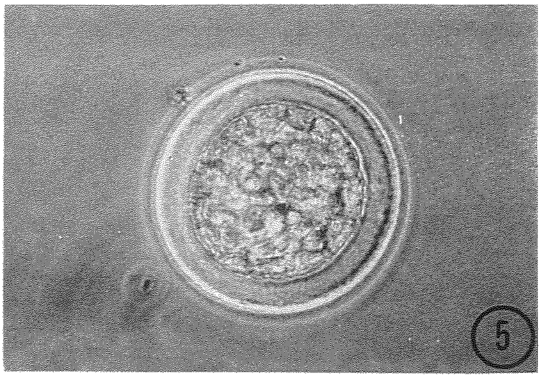
PLATE 1

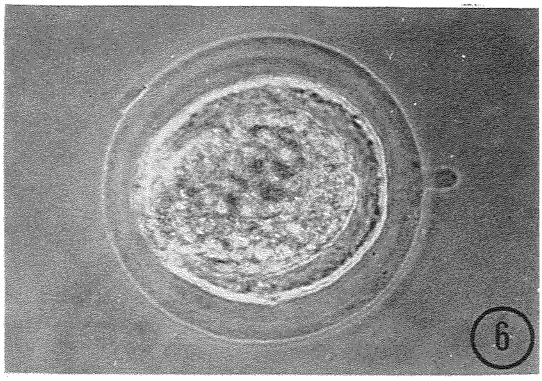


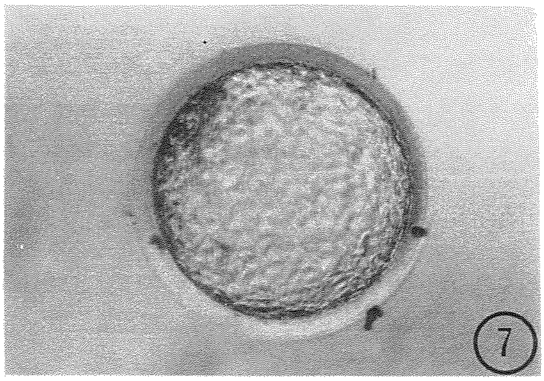












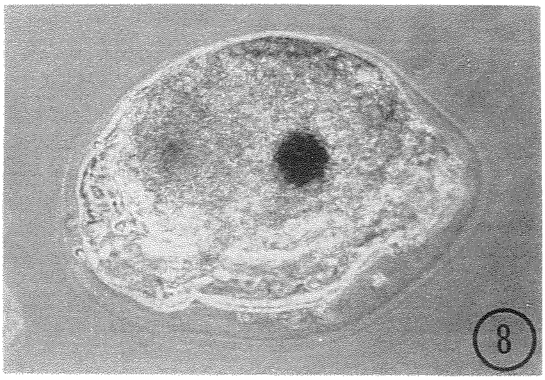


Plate 2

Explanation of figures

Fig. 9. A degenerating blastocyst recovered 106 hours *post coitum*. (Group V). ×174.

Fig. 10. A degenerating blastocyst recovered 118 hours *post coitum*. Vitelline material was liberating from the zona pellucida. (Group III). ×77.

Fig. 11. An empty zona pellucida recovered 118 hours *post coitum*. (Group III). ×77.

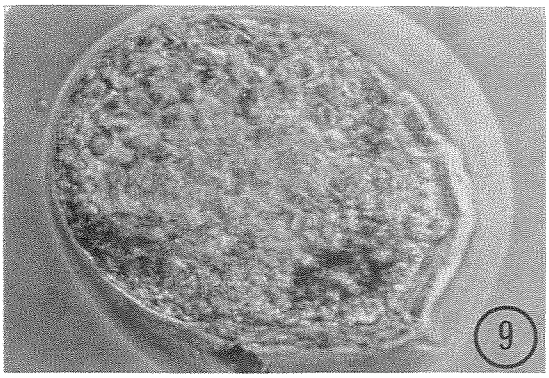
Fig. 12. A degenerating blastocyst recovered 118 hours *post coitum*. (Group III). ×77.

Fig. 13. A degenerating blastocyst recovered 118 hours *post coitum*. (Group III). ×77.

Fig. 14. A large empty zona pellucida recovered 154 hours *post coitum*. (Group V). ×77.

Fig. 15. A degenerating blastocyst recovered 178 hours *post coitum*. (Group V). ×77.

Fig. 16. Eggs recovered from the oviduct 178 hours *post coitum* at autopsy. (Group V). ×77.



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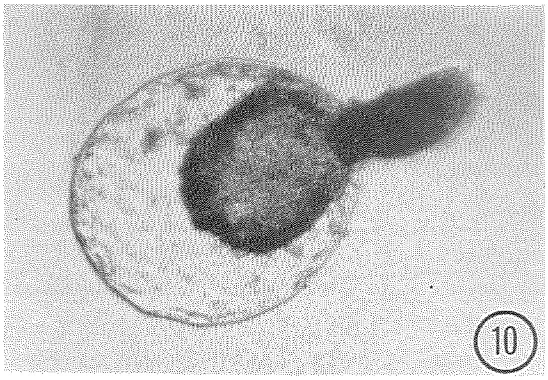

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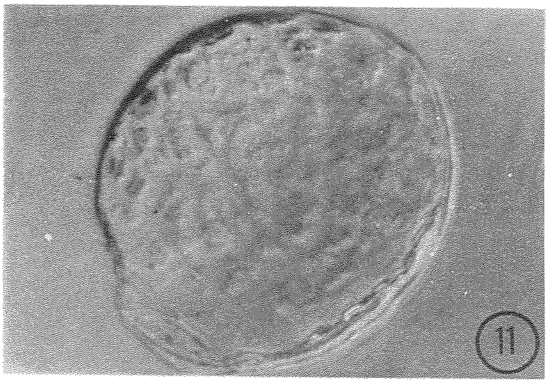

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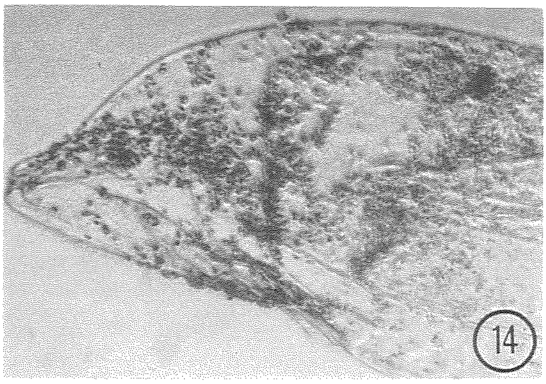

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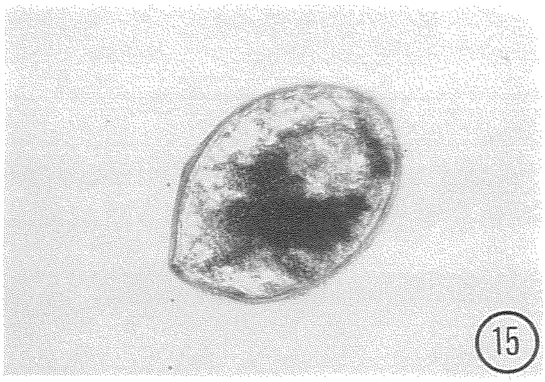

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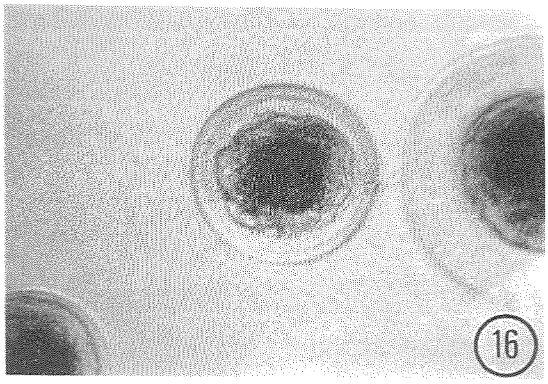

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