The transport of cumulus egg masses through the ampullae of the oviducts in the pigtailed, *Macaca nemestrina*

Transporte del cúmulo ooforo por el segmento ampular
del oviducto del mono, *Macaca nemestrina*

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The transport time of 39 freshly ovulated rabbit cumulus masses through the ampullae of the oviducts in 12 *Macaca nemestrina* was measured *in vivo* at about the time of ovulation. The mean transport time from the ostium of the infundibulum to the ampullary-isthmic junction was 22.9 ± 6.4 minutes. Freshly ovulated rabbit cumulus masses served as surrogates because their size and physical properties were determined to be remarkably similar to those of monkey cumuli recovered from preovulatory follicles near the time of ovulation. On the basis of direct observations *in vivo*, we conclude that the ovum transport through the monkey ampulla is effected primarily by ciliary action. Rhythmic peristaltic muscle contractions of the ampulla were observed and recorded. The cinematographic records were digitized to generate data on the role of muscle and ciliary activity in effecting transport time. The data revealed that muscle contractions did not significantly effect egg transport through this segment of the oviduct.

INTRODUCTION

In various mammalian species, eggs as well as ovum surrogates placed inside the Fallopian tube undergo a characteristic pattern of pro and anti-uterine pendular motions, resembling short-lived peristaltic events (Harper, 1961a, 1961b, and 1965; Talo and Brundin, 1971). Detailed measurements of egg motion and recordings of electrical activity of the circular tubal smooth muscle, strongly suggest that myoelectric activity and the mechanical performance of the myosalpinx have a strong random component. The first objective assessment of the presence of stochastic elements in ovum transport was reported at the WHO Symposium on Ovum Transport and Fertility Regulation (Verdugo *et al.*, 1976). Since that time several groups have used this approach to characterize tubal transport (Portnow *et al.*, 1977 a and b), and it is now well accepted that tubal transport should perhaps be regarded as a stochastic process (Harper, 1988). A comprehensive stochastic model for tubal transport that incorporate the role of muscle contractions and ciliary propulsion has been lately formulated (Verdugo *et al.*, 1980). However, the generalization of the notion of random tubal transport has been limited, as it has been verified only on transport measurements in the rabbit oviduct. Here we present the first detailed measurements of egg transport in the ampulla of the monkey *Macaca nemestrina*. The stochastic analysis of this data indicate that, much like in the rabbit, tubal transport in the monkey also exhibits a strong random component. In the postovulatory period, the statistical structure of egg motion reveals no apparent programming of the random peristaltic activity. Thus, ampullary transport in the *Macaca nemestrina* seems to be mainly driven by ciliary propulsion.

MATERIAL AND METHODS

Twelve healthy mature female *Macaca nemestrina* were supplied by the Regional

Primate Research Center at the University of Washington, in Seattle. They were housed either in a colony room or individual cages and were fed a vitamin-supplemented nutritionally balanced diet. Water was constantly available. The rooms were maintained at a temperature of 20° to $22^\circ C$.

Timing of the menstrual cycles

To avoid the possible effects of repeated anesthesias and laparoscopic procedures, the menstrual cyclicity was followed for several months by observing the animals several times daily and recording the incidence of vaginal bleeding. Changes in tumescence and coloration of the sex skin were used as an index of ovulation. The sex skin of the *Macaca nemestrina* gradually swells and reaches a peak of tumescence and coloration at about the midpoint of the cycle (Kuehn *et al.*, 1965; Blakely *et al.*, 1972; White *et al.*, 1974). As shown by Steiner *et al.* (1977) the mean maximum swelling of the sex skin occurs "on the same day as the estradiol 17 β and luteinizing hormone peak". In the 12 monkeys used in this study, 56 cycles of peak sex swelling were observed. The cycles varied in length from 24 to 45 days with a mean of 31.6 days. Monkeys with sex skin at the peak of swelling were classified as preovulatory and those showing the earliest signs of detumescence were classified as early postovulatory. The presence of either a large preovulatory follicle or a very recently ruptured follicle was established by careful examination of serially sectioned and stained ovaries in all 12 monkeys used in this studies.

The effect of anesthesia on muscle contractions

All observations on egg transport were made under light halothane-oxygen anesthesia (Fluothane, Ayerst Laboratories, Inc., New York, NY). We had ascertained in earlier studies that prolonged halothane anesthesia does not affect myosalpinx contractility. (Blandau *et al.*, 1975; Halbert *et al.*, 1975, 1976a and 1976b; Noonan *et al.*, 1978).

Procedure for observing egg transport

Transport of freshly ovulated cumulus masses from the fimbria, through the ostium and to the ampullary-isthmic junction was assessed using a modification of the procedure described previously for the rabbit (Harper, 1961 a and b: Blandau, 1971). Mid-line laparotomies were performed under light halothane-oxygen anesthesia. A thermoregulated horseshoe-shaped retractor was placed in the peritoneal cavity in such a manner as to hold the intestines out of the way and permit an unobstructed view of the uterus, oviducts and ovaries. The lower abdominal wall served as the sides of a chamber that was filled immediately upon opening with Hanks' balanced salt solution, warmed to body temperature and maintained at $37.5^\circ C$ by the thermoregulator. All vascular and neural elements of the reproductive tract were left intact.

Rabbit cumulus masses or surrogate ova

Rabbit eggs in cumulus were used in these studies since it was not feasible to obtain freshly ovulated monkey eggs. Individual cumulus masses, each containing a single egg, were flushed from the oviducts of donor rabbits 11-12 hours after induced ovulation. The cumulus masses were stained supravitally with diluted methylene blue to make them visible inside the ampullary lumen. Ampullary transport of stained eggs was followed through a dissecting microscope and recorded cinematographically. The oviducts were transilluminated by a 5 mm fiber optic bundle, using an infrared-filtered 200 Watt zirconium light source.

By this technique, the position and movement of the stained cumulus masses could be followed and filmed without interruption as they progressed through the ampullae. The cumulus masses were placed on the fimbriae by means of fine polished glass pipettes that had been treated with silicone to prevent adherence of the sticky cumulus. The time of transport from the ostium of the infundibulum to the ampullary-isthmic junction was determined by use of a stop watch. Ci-

nematographic records were made of portions of the transport in 9 of the 12 monkeys for more detailed analysis. Plots of the position of the cumulus mass within the exposed ampullae as a function of time were generated from data obtained by digitizing cinematographic records of transport in 4 animals. Instant velocities were calculated from the position plots of the cumulus mass. The statistical structure of the egg transport was characterized by the cumulative distribution function and probability density function of egg motion.

Since rabbit ovulated eggs in cumuli were to be used as surrogates we determine how closely they resembled ovulated monkey cumuli in their volume, viscoelastic properties, and stickiness. Estrogen assays were performed on several regularly cycling rhesus monkeys at the Oregon Regional Primate Center under the direction of Dr. Robert Brenner. The cumulus masses recovered by laparotomy at the peak of the estrogen production were essentially identical in its volume and physical characteristics to those recovered from ovulatory rabbit follicles.

Preparation of tissues of the ampulla

The folding pattern of the intraluminal mucosa of the pig-tailed monkey ampullae at midcycle was studied by light microscopy. Oviducts were quick-frozen in situ with freon pre-cooled in liquid nitrogen. Microphotographs were obtained from fresh frozen unfixed serial sections of the ampullae mounted on glass slides and stained in hematoxylin and eosin.

RESULTS AND DISCUSSION

It has been reported that ovum transport through the entire oviduct of the rhesus monkey requires 3 or 4 days (Mastroianni et al., 1967; Marston et al., 1969 a, b; Eddy et al., 1975). As far we are aware, no one has yet observed the manner of transport of ovulated cumulus masses within the ampullae of a living primate.

Jainudeen and Hafez (1973) measured the rate of egg transport in different portions of the oviducts in crab-eating ma-

caques (Macaca fascicularis). They reported that the eggs or microspheres were transported to the ampullary-isthmic junction within 48 hours, microspheres were retained within the isthmus between 48 and 56 hours, and entered the uterus approximately 96 to 120 hours after ovulation. Normally, freshly ovulated monkey eggs are surrounded by a sizeable and sticky cumulus oophorus as they are transported from the ovary to the ampullary-isthmic junction. By the time they enter the isthmus, 24 or more hours later, the cumulus has been largely dispersed (Eddy et al., 1975).

The sectioning and flushing methods used in the past to study tubal transport have limited value for the detailed investigation of egg transport/timing through the different segments of the tube. In our experience, denuded eggs or smooth microspheres will not retain their original position within the oviductal lumen if the mesosalpinges are first trimmed away and the oviducts are stretched and straightened and then divided for flushing.

A more reliable technique to time ovum transport within the oviducts in Macaca mulatta was developed by Eddy et al. (1975, 1976) and in the baboon (Papio anubis). Laparoscopic examinations at 12 to 24 intervals to confirm ovulation were conducted following the estrogen peak. When ovulation had occurred, midventral laparotomies were performed and 30 gauge needles were inserted into various oviductal subdivisions in situ. The oviductal segments were then flushed with saline to recover the unfertilized eggs. Using this method the authors concluded that in the baboon and the macaca the unfertilized ova remain for equal lengths of time in the ampullae and isthmus (Eddy et al., 1975, 1976).

Although it is important to know the time course of egg transport through the reproductive tract, this information alone will not answer the question as to how egg transport is effected through each segment of the genital tract. The involvement of cilia, the smooth muscle and the hydrodynamics of the luminal fluids must all be considered as potentially contributing to gamete transport.

The rabbit and the rat are the only animals in which there have been direct *in vivo* observations on how the ovulated eggs in cumulus are transported from the ostium to the ampullary-isthmic junction (Harper, 1961a, 1961b, 1965; Boling and Blandau, 1971; Blandau, 1969, 1978). In both of these species, ovulated eggs in cumuli take less than ten minutes to travel from the ostium to the ampullary-isthmic junction (Blandau, 1978). Direct visualization of eggs, embedded in their cumuli, moving through the ampullae of ovulating rabbits reveals a complex pattern of segmented peristaltic contractions. This striking pattern gives an impression that the ovulated cumulus masses are "milked" forward. Although it has been recognized for some time that cilia are active in the transport of ova, especially over the fimbria and through the first few millimeters beyond the ostium, their participation in egg transport through the remaining ampullae has not received the attention they deserve.

In the present experiments the mean time required for the transport of 39 cumulus masses from the os of the infundibulum to the ampullary-isthmic junction was 22.9 ± 6.4 minutes. There was no significant difference in the transport time between the midcycle females that had not yet ovulated (preovulatory, 6 animals) and those that were immediately postovulatory (6 animals).

When freshly ovulated rabbit eggs in cumulus were placed on the fimbrial surface of the monkey oviduct, they were transported toward the os of the infundibulum by ciliary action. Cumulus masses placed anywhere on the fimbrial surface were transported to the os, suggesting that ciliary flow probably converges toward the infundibulum.

Previous studies have shown that at midcycle at least 50% epithelial cells of the fimbria are ciliated (Gaddum-Rosse et al., 1973; Rummery et al., 1978). Although monkey cilia do not have the filamentous glycocalyx or "ciliary crowns" observed on the tips of the cilia in several rodents (Anderson and Hein, 1977; Dirksen and Satir, 1972), they can readily drive ovum transport through the fimbrial surface.

At about the time of ovulation the mucosal folds of the ampulla almost completely fill the lumen (Fig. 1). Because of the complexity and close apposition of the ciliated mucosal folds, the cumulus mass moving through it is in continuous and intimate contact with the beating cilia. At least 50% of the surface cells of the ampullary mucosa are ciliated and all the cilia beat toward the ampullary-isthmic junction (Gaddum-Rosse et al., 1973; Gaddum-Rosse and Blandau, 1976; Rumery et al., 1978).
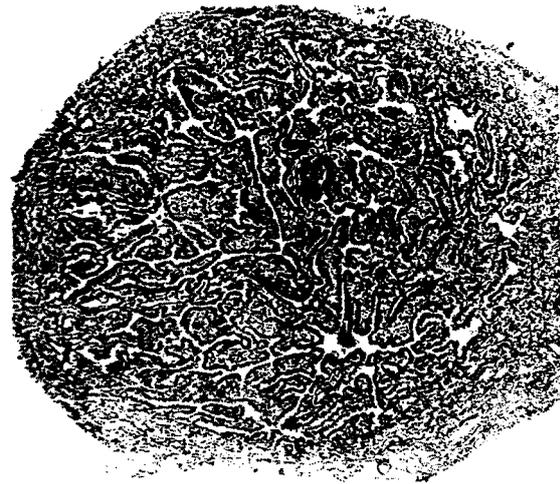
Fig. 1: Ink drawing of a hematoxylin and eosin stained, frozen section through the mid-ampulla of a *Macaca nemestrina* oviduct removed at midcycle. Note the complexity and extent of the mucosal folds and the fact that there is only a potential lumen. Magnification 50X.

Surrogate cumulus masses appear elongated as they are pulled by the cilia into the first few millimeters of the ampullae. Thereafter, its shape changes constantly as it is molded by the action of muscle contractions and the cilia lining the irregular mucosal folds.

The characteristic pendular pattern of ampullary egg motion reported in other species was also observed in the pig-tailed monkey. The cumulus masses exhibited back and forward as well as lateral motions inside the ampullary lumen. Figure 2 shows a typical plot of the position of the cumulus mass along the axial projection of the ampulla, as a function of time. The average transport rate measured from the time/distance plots was 33.8 ± 7.0 μm/

second (Mean ± SEM). Notice that the projected axial net displacements of the egg are smaller than those observed in the rabbit. However, the velocities calculated from the total three dimensional movement of the egg in the tube were quite similar to those measured in the rabbit (100-130 μm/sec. Verdugo et al., 1980). This apparent discrepancy can be explained, because in the monkey, the ovum spend a substantial amount of time moving diagonally or laterally inside the convoluted ampullary lumen. At the ampullary-isthmic junction the eggs were slowly rotated, undergoing continuous changes in shape and position, but did not move into the isthmus.
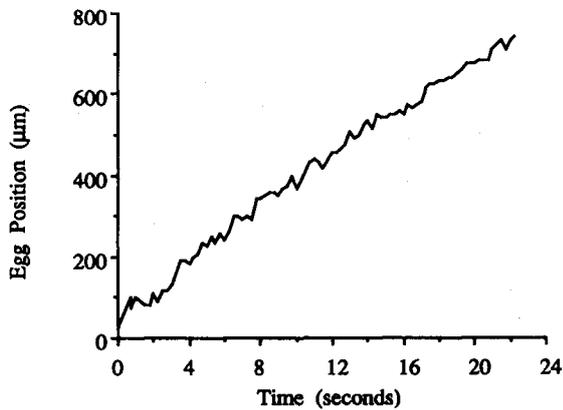
Fig. 2: Graphical representation of the position of a rabbit cumulus mass within the monkey ampullae as a function of time. The average transport rate is 33.8 ± 7.0 μm/second (Mean ± SEM). The transport of the egg in cumulus is almost continuous with small random pendular movements induced by muscle contractions.

Previous investigations have indicated that ampullary ovum transport can be described as a Langevinian process (Verdugo et al., 1980). Egg motion results from the interaction of both random and non-random driving forces produced by myosalpinx contractions and ciliary activity respectively. (Verdugo et al., 1980). This model is further supported by the observation that tubal transport can occur in the absence of myosalpinx contractions (Halbert et al., 1976b).

The relative contribution of muscle contractions and ciliary propulsion can be readily evaluated by investigating the

statistical structure of the egg motion (Verdugo et al., 1976). For instance, a shifted Gaussian pattern of egg velocities suggests that the muscle contraction lack directional programming, and thus, net pro-uterine ovum transport is driven by ciliary motion. Conversely, a skewed Gaussian probability density function suggests some degree of directional programming in the myosalpinx contractility that could provide net pro-uterine bias to the egg transport. Thus, according to this model the neuro-hormonal influences could regulate tubal transport by either modulating ciliary activity or else by controlling the programming of myosalpinx contractility.

In the present experiments, the probability density function of egg velocities, measured from the position plots, exhibited a characteristic Gaussian shape. It was centered at 33.8 μm/sec in a pro-uterine direction, and did not show any significant skewness value ($g = -0.059$; $p > 0.10$; Fig. 3). Thus, the stochastic analysis of monkey ampullary transport suggests that like in the rabbit, the myosalpinx contractions result in a pattern of random

Velocity μm/sec
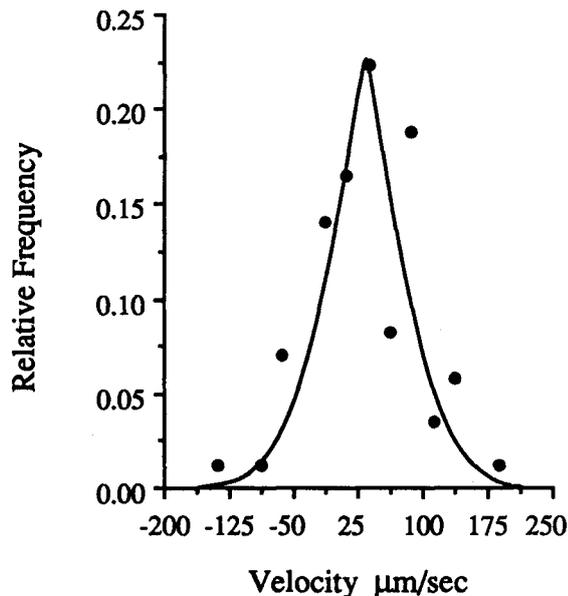
Fig. 3: Probability density function of egg velocities, measured from the position plots of rabbit cumuli in the ampullae of *Macaca nemestrina*. Note the characteristic Gaussian shape centered at 33.8 μm/sec in a pro-uterine direction. No statistically significant skewing is observed ($g = -0.059$; $p > 0.10$).

bidirectional motions without any apparent skewness that would suggest some degree of directional programming. Net transport probably results from the pro-uterine bias imposed by continuous ciliary action. However, the present observations do not rule out the possibility that in other periods of the cycle, or under different hormonal influences, muscle contractions might be programmed to bring about net directional tubal transport.

We conclude from these experiments that the slow, steady progress of the cumulus mass appears to take place quite independently of muscle contractions, and that the primary mechanism of tranport in the ampulla of the *Macaca nemestrina* is driven by ciliary activity.

ACKNOWLEDGEMENTS

REFERENCES

ANDERSON, R.G.W.; HEIN, C.E. (1977) Distribution of anionic sites on the oviduct ciliary membrane. *J. Cell. Biol. 72:* 482-492.

BLAKELY, G.A.; MORTON, W.R.; SMITH, O.A. (1972) Husbandry and breeding of *Macaca nemestrina.* Proceedings of the 3rd Conference on Experimental Medicine and Surgery in Primates; Lyon. Part 1: 61-72.

BLANDAU, R.J. (1969) Gamete transport: Comparative aspects. In: Hafez, E.S.E.; Blandau, R.J. (eds.) *The Mammalian Oviduct.* Chicago: University of Chicago Press; pp. 129-162.

BLANDAU, R.J. (1971) Observing ovulation and egg transport. In: Daniel, J.C., Jr..; Freeman, W.H. (eds.) *Methods in Mammalian Embryology.* W.H. Freeman. San Francisco; pp. 1-14.

BLANDAU, R.J. (1978) Mechanism of tubal transport: Comparative aspects. In: Brosens, I.S.P.; Winston, R. (eds.) *Reversibility of Female Sterilization.* New York: Academic Press; pp. 1-20.

BLANDAU, R.J.; BOLING, J.L.; HALBERT, S.A.; VERDUGO, P. (1975) Methods for studying oviductal physiology. *Gynecol Invest 6:* 123-145.

BOLING, J.L.; BLANDAU, R.J. (1971) Egg transport through the ampullae of the oviducts of rabbits under various experimental conditions. *Biol. Reprod. 4:* 174-184.

DIRKSEN, E.R.; SATIR, P. (1972) Ciliary activity in the mouse oviduct as studied by transmission and scanning electron microscopy. *Tissue. Cell. 4:* 389-404.

EDDY, C.A.; GARCIA, R.G.; KRAEMER, D.C.; PAUERSTEIN, C.J. (1975) Detailed time course of ovum transport in the rhesus monkey (*Macaca mulatta*). *Biol. Reprod. 13:* 363-369.

EDDY, C.A.; TURNER, T.T.; KRAEMER, D.C.; PAUERSTEIN, C.J. (1976) Pattern and duration of ovum transport in the baboon (Papio anubis). *Obstet. Gynecol. 47:* 658-664.

GADDUM-ROSSE, P.; BLANDAU, R.J. (1976) Comparative observations on ciliary currents in mammalian oviducts. *Biol. Reprod. 14:* 605-609.

GADDUM-ROSSE, P.; BLANDAU, R.J.; THIERSCH, J.B. (1973) Ciliary activity in the human and *Macaca nemestrina* oviduct. *Am. J. Anat. 138:* 269-275.

HALBERT, S.A.; BOLING, J.L.; BLANDAU, R.J.; RINGO, J.A. (1975) An optoelectronic instrument for chronic monitoring of oviduct contractions. Conference on Engineering in Medicine and Biology 17: *313 Abst.*

HALBERT, S.A.; STEGALL, H.F.; RINGO, J.A ; CANFIELD, D.R.; BLANDAU, R.J. (1976a) An optoelectronic instrument for ovulation detection in animals. *Gynecol. Invest. 7:* 363-376.

HALBERT, S.A.; TAM, P.Y.; BLANDAU, R.J. (1976b) Egg transport in the rabbit oviduct: the roles of cilia and muscle. *Science 101:* 1052-1053.

HARPER, M.J.K. (1961a) Egg movement through the ampullar region of the fallopian tube of the rabbit. Proceedings of the IVth International Congress on Animal Reproduction; The Hauge. pp. 375-380.

HARPER, M.J.K. (1961b) The mechanisms involved in the movement of newly ovulated eggs through the ampulla of the rabbit fallopian tube. *J. Reprod. Fertil. 2:* 522-524.

HARPER, M.J.K. (1965) Transport of eggs in cumulus through the ampulla of the rabbit oviduct in relation to day of pseudopregnancy. *Endocrinology. 77:* 114-123.

HARPER, M.J.K. (1988) Gamete and zygote transport. In: Knobil, E.; Neill, J.D. (eds.) *The Physiology of Reproduction,* Vol. 1. New York: Raven Press; pp. 103-134.

JAINUDEEN, M.R.; HAFEZ, E.S.E. (1973) Egg transport in the macaque (*Macaca fascicularis*). *Biol. Reprod. 9:* 305-308.

KUEHN, R.E.; JENSEN, G.D.; MORRILL, R.K. (1965) Breeding *Macaca nemestrina:* a program of birth engineering. *Folia. Primatol. 3:* 251-262.

MARSTON, J.H.; KELLY, W.A.; ECKSTEIN, P. (1969a) Effect of an intrauterine device on gamete transport and fertilization in the rhesus monkey. *J. Reprod. Fertil. 19:* 149-156.

MARSTON, J.H.; KELLY, W.A.; ECKSTEIN, P. (1969b) Effect of an intrauterine device on uterine mobility in the rhesus monkey. *J. Reprod. Fertil. 19:* 321-330.

MASTROIANNI, L, JR.; SUZUKI, S.; MANABE, Y.; WATSON, F. (1967) Further observations on the influence of the intrauterine device on ovum and sperm distribution in the monkey. *Am. J. Obstet. Gynecol. 99:* 649-660.

NOONAN, J.J.; ADAIR, R.L.; HALBERT, S.A.; RINGO, J.A.; REEVES, J.J. (1978) Quantitative assessment of oxytocin-stimulated oviduct contractions of the ewe by optoelectronic measurements. *J. Anim. Sci. 47:* 914-918.

PORTNOW, J.; TALO, A.; HODGSON, B.J. (1977a) A random walk model of ovum transport. *Bull. Mathem. Biol. 39:* 349-357.

PORTNOW, J.; HODGSON, B.J.; TALO, A. (1977b) Simulation of oviductal ovum transport. *Can. J. Physiol. Pharmacol. 55:* 972-974.

RUMERY, R.E.; GADDUM-ROSSE, P.; BLANDAU, R.J.; ODOR, D.L. (1978) Cyclic changes in ciliation of the oviductal epithelium in the pig-tailed macaque (*Macaca nemestrina*). *Am. J. Anat. 153:* 345-366.

STEINER, R.A.; SCHILLER, H.S.; ILLNER, P.; BLANDAU, R.J.; GALE, C.C. (1977) Sex hormones correlated with sex skin swelling and rectal temperature during the menstrual cycle of the pigtail macaque (*Macaca nemestrina*). *Lab. Anim. Sci. 27:* 217-221.

TALO, A.; BRUNDIN, J. (1971) Muscular activity in the rabbit oviduct: A combination of electric and mechanic recordings. *Biol. Reprod. 5:* 67-77.

VERDUGO, P.; BLANDAU, R.J.; TAM, P.Y.; HALBERT, S. (1976) Stochastic elements in the development of deterministic models of egg transport. In: Harper, M.J.K.; Pauerstein, C.J.; Adams, C.E.; Coutinho, E.M.; Croxatto, H.B.; Paton, D.M. (eds.) *Ovum Transport and Fertility Regulation.* World Health Organization Symposium. Copenhagen: Scriptor; pp. 126-137.

VERDUGO, P.; LEE, W.I.; HALBERT, S.; BLANDAU, R.J.; TAM, P.Y. (1980) A stochastic model for oviductal egg transport. *Biophys. J. 29:* 257-270.

WHITE, R.J.; BLAINE, C.R.; BLAKELY, G.A. (1973) Detecting ovulation in *Macaca nemestrina* by correlation of vaginal cytology, body temperature and perineal tumescence with laparoscopy. *Am. J. Phys Anthropol 38:* 189-194.