associated with sewage (streptococcal infections and tuberculosis), no significant differences in disease incidence were seen. This observation tends to rule out biased reporting by the settlements, since faulty reporting should have brought to light a distinct difference in the morbidity ratio of these diseases.

During the winter months (December to March) sewage irrigation is not practiced. If there is a connection between sewage irrigation and an increased incidence of enteric disease, the winter period should show no difference between the sewage and tap-water users. We also collected the data for the winter months, but because of the low morbidity rate, information could be obtained only for shigellosis and infectious hepatitis (Fig. 1, winter). No significant differences were found in disease rate between both groups of kibbutzim. This strengthens the hypothesis that there is a relationship between irrigation with sewage effluent and enteric disease incidence.

This retrospective study provides some epidemiologic evidence for an increased risk of enteric communicable diseases among the utilizers of wastewater. The fact that no significant differences are reported for diseases not considered to be associated with sewage or for enteric diseases during the nonirrigation period supports the assumption that reporting is essentially uniform for both groups.

The quality of the drinking water in all communities studied is good and is monitored routinely both by the water supply company and the Ministry of Health. The possibility of pathogen transmission by sewage-irrigated crops has been discounted since Ministry of Health regulations do not allow the use of sewage for the irrigation of vegetables or other crops consumed raw. Geographic factors have been discounted since settlements in both groups are distributed more or less uniformly in all areas of the country.

These findings indicate that the health hazards associated with partially treated nondisinfected wastewater irrigation may be greater than previously assumed. In the case of the kibbutzim studied, the areas spray-irrigated with wastewater were 100 to 3000 m from the residential areas. No direct evidence is available at this time concerning the actual concentration of pathogens in the air at the residential areas, although studies have shown that enteric bacteria of sewage origin could be detected as far as 1200 m from the site of a sewage trickling filter plant (*6*). It is also possible that the pathogens from the wastewater irrigation areas can reach the kibbutz populations by an alternate pathway, on the bodies and clothes of the irrigation workers who live in the community and return from the fields at mealtime and at the end of the day.

The introduction of a high degree of wastewater treatment, including effective inactivation of bacterial and viral pathogens by disinfection of all wastewater utilized for any purpose in the vicinity of settlements or residential areas, would appear to be a reasonable precaution in light of these findings.

ELIYAHU KATZENELSON
ITZHACK BUIUM
HILLEL I. SHUVAL
Environmental Health Laboratory, Hebrew University–Hadassah Medical School, Jerusalem, Israel

References and Notes

1. J. L. S. Hickey and P. C. Reist, *J. Water Pollut. Control Fed.* **47**, 2741 (1975); *ibid.*, p. 2758.
2. C. S. Clark, E. J. Cleary, G. M. Schiff, C. C. Linnemann, Jr., J. P. Phair, T. M. Briggs, *J. Environ. Eng. Div. Am. Soc. Civ. Eng.* **102**, 375 (1976).
3. C. A. Sorber, S. A. Schaub, H. T. Bausam, in *Virus Survival in Water and Wastewater Systems*, J. F. Malina, Jr., and B. P. Sagik, Eds. (Center for Research in Water Resources, University of Texas, Austin), pp. 241–252; C. A. Sorber, H. T. Bausam, S. A. Schaub, in *Proceedings of the 1975 Sprinkler Irrigation Technical Conference* (Sprinkler Irrigation Association, Silver Spring, Md., 1975), pp. 204–214.
4. E. Katzenelson and B. Teltch, *J. Water Pollut. Control Fed.* **48**, 710 (1976).
5. J. O. Ledbetter, L. M. Hauck, R. Reynolds, *Environ. Lett.* **4**, 225 (1973).
6. A. P. Adams and J. C. Spendlove, *Science* **196**, 1218 (1970).
7. Supported in part by grants from the Rockefeller Foundation and the Office of the Water Commissioner, Ministry of Agriculture, Israel. The assistance given by the staff of the Ministry of Health in gathering the communicable disease data is gratefully acknowledged, as is the advice on questions of epidemiology provided by Dr. M. Davies.

28 July 1976

Ovum Aging and *p*H Imbalance as a Cause of Chromosomal Anomalies in the Hamster

Abstract. *Using female hamsters mated in estrus, we have produced triploid embryos as manifestations of pregnancy wastage by combining delays of 3 to 4 hours in fertilization with exposure of the animals to hypoxia. Among the triploid embryos only XXX and XXY karyotypes occurred, indicating derivation from XX-containing ova. These findings have relevance to human reproduction.*

Experiments conducted in the 20-year period 1935 to 1955 documenting the adverse effects of fertilization delays on reproduction in rats, mice, guinea pigs, and rabbits were summarized by Chang and Fernandez-Cano (*1*). Shortly before this study appeared, Tjio and Levan had demonstrated 46 chromosomes in man (*2*), in a study that initiated a decade of investigations aimed at adapting tissue culture techniques to the demonstration of animal karyotypes (*3*) including human aneuploidies.

In hamsters it has been found that hypoxia (*4*), delayed fertilization (*5*), maternal aging (*6*), and *p*H equilibrium (*6, 7*) may each, acting alone or interdependently, have a decisive influence on the occurrence of chromosomal polyploidies and aneuploidies (*6*). Female hamsters exposed to hypoxic atmospheres in the postcoital period conceive many embryos with triploid and tetraploid karyotypes (*4*). Chromosomal aneuploidies and other manifestations of pregnancy wastage are evident in the fetuses of mothers subjected to delays of fertilization. Nine hours after the estimated time of ovulation, sexual activity of the female hamster markedly diminishes, and mating, even when it takes place, seldom results in fertilization. Even a delay as short as 3 hours beyond the estimated time of ovulation results in measurable increases of pregnancy wastage, that is, in decreased yields of normal embryos, increased embryonic mortality, and in aneuploid karyotypes of surviving embryos examined after 9 days of intrauterine development (*5*).

The importance of *p*H balance (7.1 to 7.3) for normal mitosis, and the mutagenic effect of *p*H disturbances on cell division, has been demonstrated in vitro with human blood cultures (*7*). More recently, by using critical *p*H disturbances accompanying hypoxia as a mutagenic agent, an interdependent action of maternal aging and *p*H disturbances on chromosomal patterns of 9-day-old embryos was demonstrated (*6*). This last finding led us to examine the possibility of there being a similar interdependency between postovulatory aging and *p*H imbalance.

Healthy female Syrian hamsters (*Mesocricetus auratus*) aged 3 to 6 months were used in these experiments. They were housed in separate cages, kept under conditions of reversed lighting (6:00 p.m. to 5:59 a.m.) (*1*), and darkness (6:00 a.m. to 5:59 p.m.) for 3 weeks so that es-

trus would occur during customary working hours. Estrous cycles were identified by vaginal exudate around 9:00 a.m. daily and the stage of early estrus was confirmed by eliciting the characteristic lordotic response which female hamsters show when exposed to males. After 3 to 4 weeks, these animals had adapted to artificial daylight; the changed 4-day estrous cycles had become regular and the animals were ready to use. Since all animals were young adults and had not been bred before, the possibility that maternal aging would influence experimental design did not arise.

With the variable of maternal age reasonably well excluded by the simple expedient of using only young adults as breeders, the two major factors remaining to be adjusted were ovum aging, that is, the time between ovulation and fertilization, and the degree of *p*H imbalance, that is, the time and timing of hypoxic stress. We considered that ovum aging began at ovulation, or more accurately with reference to this particular experiment, at the estimated time of ovulation, this time being early (around 2:00 to 3:00) in the afternoon rather than around dawn (2:00 to 3:00 a.m.), as is the case naturally. Control animals (subjected to the same cycles of light and darkness as the experimental animals) were mated under normal conditions about 5 hours before the estimated time of ovulation. The length of fertilization delay was determined directly by the timing of copulation. The severity of *p*H imbalance was determined indirectly by the duration (3 to 4 hours) and severity of hypoxia [the animals were subjected to an oxygen pressure equivalent to that found at an altitude of 30,000 feet (9400 m)].

Fifty females were divided into three groups (see Table 1). Group 1 consisted of 12 controls; group 2 consisted of 11 copulated females that were used to provide baseline values for pregnancy wastage after copulation delays of 3 to 4 hours beyond the estimated time of ovulation; and group 3 consisted of 27 females that were used to quantitate the combined effect of being subjected to both stresses (hypoxia and delayed fertilization) simultaneously. Group 3 animals thus were submitted to low pressure hypoxia designed to induce *p*H imbalance at the very time when ovum aging was reaching a critical point.

Pregnant hamsters were killed after 9 days of gestation and the uterus, embryos, and adnexa were removed and placed promptly in normal saline. The numbers of corpora lutea, implantation sites, runts, and normal embryos were counted, and then specimens were prepared for chromosomal examination according to a method that does not require prolonged culture of specimens (5).

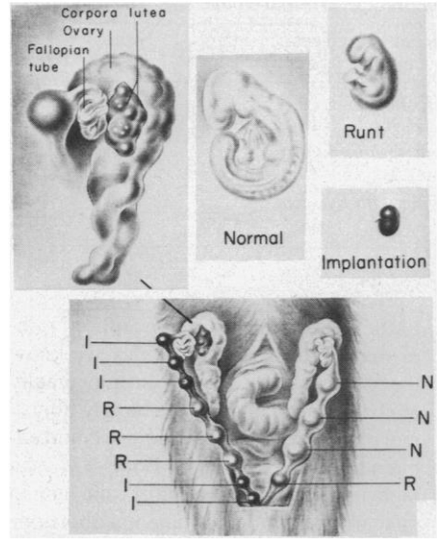

Fig. 1. Surgical exposure of uterus of a hamster 9 days after it was mated. Three triploid runts (*R*) are present in the right uterine horn, in a row flanked by three dead embryos (top) and two dead embryos (bottom) (*I*, implantation). One triploid embryo is present in the left horn (bottom) surmounted by three normal (*N*) embryos.

The data in Tables 1 and 2 indicate component parts of pregnancy wastage, such as missing zygotes (discrepancies between corpora lutea and implantations), dead and anomalous (aneuploid, polyploid), and teratologic (congenitally malformed; cytogenetically normal) embryos. In these experiments, fertilization delays of 3 to 4 hours were followed by a sharp increase in percentages of dead and anomalous embryos above control values, whereas the average number of live embryos produced per female decreased by almost 50 percent. Although we could not prove that hypoxia and ovum aging had a synergistic effect, we could measure the combined impact of these factors in terms of pregnancy wastage. The incidence of dead embryos rose significantly from 5.7 percent ($P < .001$) in controls to 17.8 percent after ovum aging and to 34.0 percent after the double stress of ovum aging and exposure to hypoxia. The data in Table 2 indicate that such stress has a cytogenetic impact on living embryos. Chromosomal anomalies that were rarely present (in less than 2 percent of controls) numbered seven triploid formations and one mosaic formation (12.5 percent anomalous) among 64 living embryos of doubly stressed mothers, four of them in a single litter (Fig. 1). The chromosomes of all triploid embryos had XXX or XXY configurations; no XYY karyotype was found. All triploid embryos appeared to be runts (Fig. 1), with crown to rump lengths averaging 2.22 mm, whereas 4.08 mm would be the expected length after 9 days of gestation.

Our data in Table 1 compare with those reported previously (5) in which no triploid karyotypes were found among 135 control embryos. We found one triploid (0.6 percent) among 148 controls. In that same experiment (6), five (4.4 percent) triploid karyotypes (none of them XYY) were found among 114 embryos produced by female hamsters prevented from mating for 3 hours after the estimated time of ovulation. We observed two triploid karyotypes (5.4 percent) among 37 embryos recovered from group 2 mothers subjected to ovum aging only. Seven triploid karyotypes (10.9 percent) were found among the 64 embryos produced by mothers submitted to the double stress of ovum aging and hypoxia.

The most interesting finding in this study was the clustered occurrence of four of the nine triploid embryos in the uterine horns of the same mother (Fig. 1) that had undergone a double stress [4-hour (estimated) delay in fertilization

Table 1. Effect of delayed fertilization with or without exposure to hypoxia on pregnancy wastage and embryonic polyploidy. Group 1 females were mated about 5 hours before the estimated time of ovulation; group 2 females were mated about 3 to 4 hours after the estimated time of ovulation; group 3 females were exposed to hypoxia, then mated about 3 to 4 hours after the estimated time of ovulation.

Number of females mated	Pregnant females		Average numbers per female		
	Number	Percent	Corpora lutea	Implantations	Live embryos on day 9
		Group 1: Controls			
12	10	33	19.1	16.0	16.0
		Group 2: Ovum aging without hypoxia			
11	4	36	18	11.3	19.3
		Group 3: Ovum aging with hypoxia			
27	9	33	17.3	11.7	8.0

Table 2. Effect on the embryos of delayed fertilization with or without exposure to hypoxia.

Total embryos		Dead embryos		Live embryos		Normal diploid		Triploid runts	
Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
				Group 1: Controls					
159	100	9	5.7	150	94.3	148	93.1	1	0.6
				Group 2: Ovum aging without hypoxia					
45	100	8	17.8	37	82.2	34	75.5	2	4.4
				Group 3: Ovum aging with hypoxia					
97	100	33	34.0	64	65.9	56	57.7	7	7.2

with 4 hours of exposure in the low-pressure chamber before copulation]. Three of the runts were clustered together in a row between two sets of implantation sites (representing dead embryos) located two in a row and three in a row at the proximal and distal ends of the right uterine horn. We infer that all eight of the as yet unfertilized ova stemming from the right ovary had been critically injured as a batch before, during, or after the 4 hours spent by the mother in the low pressure chamber before copulation and thus ± 4 hours before fertilization. Evidence is supplied both by the conditions of the experiment and the XX component of the triploid karyotypes. It would have been interesting to have measured the *p*H acid imbalance of the microclimate within the tube (*7*) during the second stage of meiosis and to know whether any of the dead embryos had been triploid runts. What is known is that of 16 ova discharged from two ovaries, 12 were fertilized, four ova or embryos had disappeared, four became triploid runts, and only 3 appeared grossly normal at 9 days of gestation. Moreover, the nature and timing of either component of the double stress is known to be capable of causing pregnancy wastage varying from embryonic death to cytogenetic aneuploidies and polyploidies to congenital malformations, depending on the time and timing of its action (*4–7*).

The finding of nothing but XXX and XXY karyotypes among the metaphase plates prepared from triploid embryos agrees with previous results (*5, 7*) in which no XYY karyotypes were found. Together, these findings favor the derivation of triploid karyotypes from an XX-containing ovum rather than a single Y-containing sperm or zygote; the additional 22 chromosomes of a triploid complement could develop during the second stage of meiosis or after fertilization as a result of failure to extrude a polar body.

Of probable relevance to an improved understanding of mammalian reproduction is the repeated demonstration that embryonic and fetal wastage can be related to aging of female eggs and hence to the circumstances of conception, rather than maternal aging. Today we know that "wastage" includes chromosomally abnormal as well as anatomically abnormal offspring. Thus our findings could explain the observations by Boué *et al.* of a high incidence of aneuploidy in human miscarriages and spontaneous abortions (*8*). This finding is usually interpreted as being a result of a discarding or debriding process on the part of nature of inferior ovums. We can find no evidence of a discard process in the hamster model; on the contrary, normal, that is, physiologic mating and conception in controls is associated with normal karyotypes in the progeny.

KIMIKO ADACHI
THEODORE H. INGALLS
Epidemiology Study Center, Framingham Union Hospital, Framingham, Massachusetts 01701

References and Notes

1. M. C. Chang and L. Fernandez-Cano, *Anat. Rec.* **132**, 307 (1958).
2. J. H. Tjio and A. Levan, *Hereditas* **42**, 1 (1956).
3. T. C. Hsu and K. Benirschke, *An Atlas of Mammalian Chromosomes* (Springer, New York, 1967).
4. T. H. Ingalls and M. Yamamoto, *Arch. Environ. Health* **24**, 305 (1972).
5. T. H. Ingalls, T. Shimada, M. Yamamoto, *ibid.* **31**, 153 (1976).
6. M. Yamamoto and T. H. Ingalls, *Science* **176**, 518 (1972).
7. T. Shimada and T. H. Ingalls, *Arch. Environ. Health* **30**, 196 (1975).
8. A. Boue and C. Thibault, Eds. *Chromosomal Errors in Relation to Reproductive Failure, Proceedings of the Symposium*, Paris, Centre International de l'Enfance, 12–14 September, 1973.

7 May 1976; revised 4 August 1976

Relaxin: A Product of the Human Corpus Luteum of Pregnancy

Abstract. *Plasma samples from peripheral and ovarian veins were obtained from women at cesarean section. A peptide that immunologically cross-reacts with a specific antiserum to porcine relaxin is present in all samples. Its concentration is four times higher in the ovarian vein draining the ovary, which contains the corpus luteum of pregnancy, than in either the peripheral vein or the contralateral ovarian vein. Secretion of ovarian relaxin correlates with secretion of ovarian progesterone, thus providing another index of luteal function.*

Relaxin is a peptide hormone identified in female pigs, guinea pigs, rabbits, mice, and rats during pregnancy (*1*). Its reported actions include cervical dilatation and softening, inhibition of uterine contractions, and relaxation of the pubic symphysis and other pelvic joints (*2*). The placenta and uterus have been suggested as possible sources of relaxin, but the major source of the hormone is the corpus luteum of the pregnant sow (*3*). The presence of relaxin in human pregnancy has been reported (*4*), but specific methods for measuring plasma levels were unavailable. Crisp and co-workers have shown that the corpus luteum of human pregnancy has the ultrastructural apparatus necessary to secrete peptide hormones as well as steroid hormones (*5*). However, to date, only hormones of ster-

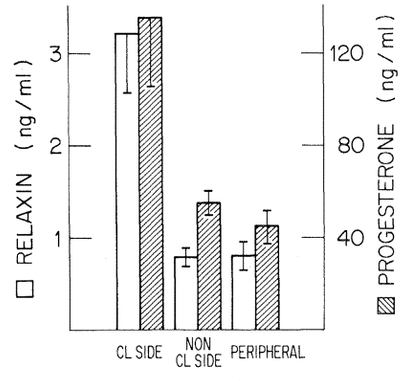

Fig. 1. Concentrations of progesterone and relaxin in ovarian vein and peripheral plasma after term delivery by cesarean section. Vertical lines represent the standard error of the mean. CL, corpus luteum.

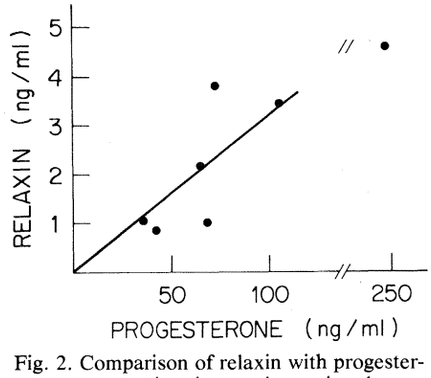

Fig. 2. Comparison of relaxin with progesterone concentration in ovarian vein plasma. Peripheral plasma concentrations have been subtracted from each sample to correct for possible nonluteal contributions.