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.

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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-

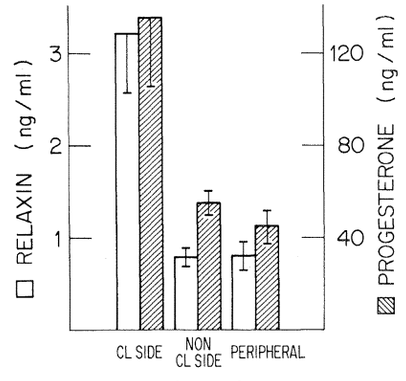

Fig. 1. Concentrations of progesterone and relaxin in ovarian vein and peripheral plasma after term delivery by cesarean section. Vertical lines represent the standard error of the mean. CL, corpus luteum.

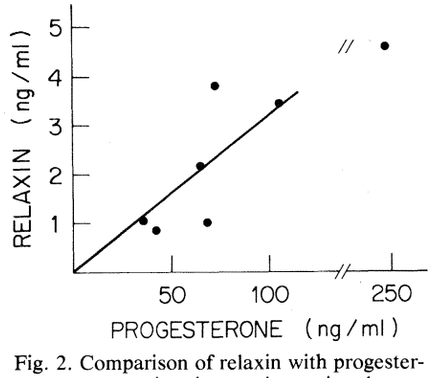

Fig. 2. Comparison of relaxin with progesterone concentration in ovarian vein plasma. Peripheral plasma concentrations have been subtracted from each sample to correct for possible nonluteal contributions.

oidal nature have been shown to be secreted by the human corpus luteum.

A specific radioimmunoassay (RIA) for porcine relaxin has been developed with ^{125}I-labeled polytyrosylrelaxin (*6*). Its antiserum was found to cross-react with relaxin-like substances in serums of many species including those of humans in late pregnancy (*7*). Using this relaxin RIA (*7*), we attempted to determine the source of human relaxin in late pregnancy.

The subjects of the study were seven pregnant women at term being delivered by cesarean section; plasma samples from both ovarian veins were obtained from each of these women 20 to 30 minutes after the placenta was delivered. The right ovarian vein was always sampled first and a peripheral blood sample was simultaneously obtained. The corpus luteum was on the right side in four women and on the left side in the other three women. All ovarian vein samples were taken with syringe and needle after the utero-ovarian blood flow was excluded by digital compression to prevent cross circulation from the other ovary. The samples were assayed for relaxin. In addition, for comparison, progesterone was measured by RIA (*8*).

Plasma progesterone concentrations (Fig. 1) were higher in the ovarian vein of the corpus luteum–bearing ovary than in the peripheral plasma ($P < .025$) or the noncorpus luteum side ($P < .05$). Relaxin concentrations were likewise significantly higher in the ovarian vein of the corpus luteum–bearing ovary than in either the peripheral plasma or the plasma of the contralateral ovary ($P < .005$). When peripheral levels are subtracted from luteal side ovarian vein concentrations and relaxin was plotted again progesterone, it can be seen that increased luteal progesterone secretion was accompanied by increased relaxin secretion (Fig. 2). Roughly, an increase of relaxin (1 ng/ml) was associated with an increase of progesterone (30 to 50 ng/ml). Such concentrations of relaxin are too low to be detected by current bioassay methods.

Our studies demonstrate that a peptide is present in the serums of pregnant women at term, which competes with porcine relaxin in a specific RIA. This substance is secreted by the pregnancy corpus luteum, and its secretion correlates with luteal progesterone secretion.

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Cellulosic Microfibrils: Nascent Stages of Synthesis in a Higher Plant Cell

Abstract. *Freeze-fracturing of untreated plasma membrane and inner wall surfaces of stelar tissue in corn roots demonstrated the association of globular complexes with the ends of nascent microfibrils. It is proposed that the granule complexes associated with the outer leaflet of the plasma membrane coordinate the assembly of the cellulosic microfibrils.*

Elucidation of the mechanisms of cellulosic microfibril formation is important in understanding the dynamic aspects of plant cell wall functions. Cellulosic microfibrils constitute the structural framework of the cell wall. The integrity and form of the cell are determined, in part, by the site and pattern of microfibrillar deposition. Likewise, microfibrillar deposition and orientation must be understood in terms of the biosynthetic pathway, the role of membranes in the assembly process, and the nature of the synthetic complex itself (*1*).

In higher plants, it has been hypothesized that microfibril synthesis and assembly occur at the cell surface (*1*), but definitive proof for this is lacking. In certain algae, however, formation of microfibrils has been found in association with the plasma membrane (*2, 3*) as well as membranes of the Golgi apparatus (*4*).

The advent of the freeze-etching technique has enabled large areas of internal and external surfaces of membranes to be examined. Several studies of plant cell membranes by this technique have indicated the involvement of the plasma membrane in cellulosic microfibril synthesis (*5, 6*). Recent investigations have demonstrated that fixatives and cryoprotectants destroy plasma membrane–wall interfaces thereby not preserving the labile structures associated with cellulose synthesis. Willison (*7*) demonstrated the deleterious effects of fixatives and cryoprotectants on cell wall–plasma membrane interfaces in *Phaseolus* root tips. Recently, by means of electron microscopy, Brown and Montezinos (*3*) demonstrated linear complexes associated with growing cellulosic microfibrils in the unicellular green alga, *Oocystis*. These complexes were preserved only in cells which had been directly frozen in Freon. Because of the advantages recognized in the direct freezing of *Oocystis* cells, it became apparent that a similar approach with a higher plant cell might yield useful data on cellulose formation.

The freeze-etch study described here was conducted on stelar tissue of 3-day-old *Zea mays* cv. Burpee snowcross roots. Corn seeds were surface sterilized in 2 percent Clorox, soaked overnight, and then germinated in the dark at 26°C ± 1°C on moistened filter paper in petri plates. Just prior to use, 1.0-mm portions of the stele were removed 12 mm ± 1 mm back from the tip, placed immediately on gold specimen holders and, without any prior treatment with fixative or cryoprotectant, quickly frozen in Freon 22, maintained at liquid nitrogen temperature, then transferred to liquid nitrogen for storage. A Balzers BA 360M freeze-etch apparatus was used, and specimens were etched at −106°C for 2 minutes prior to shadowing with platinum-carbon and coating with carbon. The replicas were cleaned in Clorox and then in 75 percent H_2SO_4. They were examined with a Hitachi HU 11E-1 electron microscope.

During the freeze-etch process, biological membranes fracture in the plane of the median hydrophobic interface (*8*). At the fractured surfaces of thin, elongate cells within excised corn steles, microfibril impressions bearing pronounced terminal globules were frequently observed within discrete regions. When the inner leaflet of the plasma membrane of this tissue is torn away, the fractured face of the outer leaflet is revealed (Fig. 1). This is termed the EF face (*9*). Typical randomly scattered membrane particles are present. Microfibrillar impressions are visible as well as associated granules measuring about 160 to 200 Å.