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Dietary Zinc and Parturition in the Rat

I. Uterine Pressure Cycles

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ABSTRACT

The pattern of uterine pressure cycles following oxytocin infusion was evaluated in near-term pregnant rats as a function of dietary zinc. Pressure cycles were monitored by a pressure transducer and polygraph linked to an intrauterine water-filled latex balloon, inserted on d 19 of gestation, in place of the right cranial fetus and its placenta. Oxytocin infusion on d 22 induced labor in all five zinc-deficient rats, but the tracings revealed irregular, poorly synchronized, and/or low amplitude patterns, compared to controls. Excessive abdominal straining was required to accomplish fetal emergence in many instances. The results are interpreted as suggestive of diminished gap-junction formation.

Index Entries: Zinc; zinc deficiency; parturition; gap junctions; uterus; estrogen; zinc, and uterine pressure cycles at birth; uterine pressure cycles, pattern of following oxytocin infusion; zinc, effects on parturition.

INRODUCTION

Zinc was first reported to be required for normal parturition in the rat by Apgar (*1*). Female rats fed a semipurified diet, containing ≤1 ppm zinc, had delayed and prolonged deliveries, accompanied by excessive bleeding. After delivery, neonates and placentae were ignored. Inde-

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pendent confirmation of this effect of zinc emerged from studies on the use of rapeseed-protein concentrate, a material rich in the zinc-chelator, phytic acid (*2–5*). O'Dell et al. (*6*) noted that zinc-deficient dams also exhibited a significant decrease in body temperature and in blood pressure at, or immediately following, parturition. Zinc is apparently specific in its effects on parturition in that the syndrome was not produced by restriction of total food intake (pair-feeding) or by consumption of diets deficient in protein, thiamin, copper, or manganese (*7,8*). An impact of zinc deficit was still evident when the limitation was imposed as late as d 18 of gestation, and zinc repletion on d 19 permitted normal delivery (*9*).

Bunce et al. (*10*) evaluated plasma progesterone, estradiol, and ovarian 20α-hydroxysteroid dehydrogenase (20α-OHSDH) activity as a function of dietary zinc in pregnant rats at term. Plasma progesterone was normal at d 20 of gestation and declined at a normal rate up to 12 h prepartum (PM of d 22), but was elevated (13 nmol/L vs 6 nmol/L) on the morning of d 23, the usual delivery date in the colony. Peak values of plasma estradiol were observed on the morning of d 22 and were not affected by dietary zinc. The normal increase in ovarian 20α-OHSDH activity on the morning of d 22 was delayed by about 8 h in the zinc-deficient animals. Induction of this enzyme is one of many estrogen-directed events necessary for the smooth transition from gestation to labor. Its tardy appearance suggested the possibility that zinc is required for normal response to estrogen instructions.

In the continued exploration of the biochemical basis of this phenomenon, we have compared uterine pressure cycle changes in zinc-deficient and control rats, following adminstration of oxytocin on d 22. The results show differences consistent with either delayed or incomplete gap junction formation, another estrogen-dependent event.

MATERIALS AND METHODS

Mature virgin female Sprague–Dawley rats (VPI & SU vivarium), weighing 250–300 g, were paired with males and checked daily for the presence of vaginal plugs or sperm in a vaginal lavage. The morning on which either a plug or sperm was found was designated d 1 of pregnancy. The female rats were then housed separately in individual polypropylene cages (27 × 21 × 15 cm), containng a floor layer of chopped corn-cob absorbent, for the remainder of the experiment. The animal room was maintained at 21–23°C temperature and 65% relative humidity. Air was exchanged and filtered continuously. Lighting was regulated automatically to provide a schedule of 12 h on and 12 h off. For the first 9 d of pregnancy, all rats were allowed free access to Purina Lab Chow and distilled water. On d 10, they were randomly assigned to either a low-zinc diet (3 ppm zinc) or a control diet (40 ppm zinc) composed

of semisynthetic ingredients (*10*). Dietary zinc concentrations were verified by chemical analysis.

On d 19, the cranial fetus and placenta of the right uterine horn were removed under metofane anesthesia and replaced by a water-filled (3 mL) latex balloon (*11*), provided with a PE 20 polyethylene catheter. The right external jugular vein was cannulated with PE 50 tubing. The two catheters were threaded subcutaneously to the back of the neck and exteriorized through a small puncture in the skin. The catheters were protected by a small metal sheath made from a 14-gauge needle and sutured to the skin. At 12 noon on d 22, the catheter from the intrauterine balloon was connected to a Gould Statham pressure transducer and Grass 7D polygraph (Grass Medical Instruments, Quincy, MA). The base-line for preinfusion uterine pressure changes was established over a 30-min period. At that time, oxytocin (O-4250, Sigma Chemical Co., St. Louis, MO) administration was begun into the jugular catheter with an infusion pump. The initial rate was 0.2 mU/min. This was increased at 30-min intervals to 0.5, 1.0, 2.0, and 5.0 mU/min, and the final rate was maintained for 4 h or until all pups were delivered, whichever was the sooner.

At the conclusion of the pressure cycle measurements, dams were anesthetized with ether, bled by cardiac puncture, using heparinized syringes, and then killed by cervical dislocation. Plasma was separated and stored at $-20°C$. Uterine horns were opened longitudinally, freed of fetuses and placentae, snap frozen in dry ice, and stored at $-80°C$. Plasma was analyzed for zinc by conventional atomic absorption spectrophotometry and for progesterone by the RIA technique of Sherwood et al. (*12*). Uterine total estrogen receptor number and affinity constants were determined, as described by Pavlik and Coulson (*13*).

An additional group of rats were placed on the control diet and subjected to a sham operation on d 19 of gestation. This consisted of placing the animals under anesthesia, exposing and handling both the uterus and right jugular vein, and then closing and suturing the wound. No fetuses were removed and no catheters were implanted. These animals were allowed to deliver spontaneously and then sacrificed for analysis of plasma and uterine tissue, as noted above.

RESULTS

Mean food intake data, weight gain, and litter size are shown in Tables 1 and 2. Loss of appetite is characteristic of zinc deficiency, but it tends to be cyclic and conditioned by the severity of the deficit and other factors. If there is a severe drop in food intake, pair-fed, as well as *ad libitum*-fed, controls may be employed. In this instance, the low zinc-fed animals did show a tendency toward diminished food intake, beginning

TABLE 1
Mean Food Intake

Diet treatment	Day of gestation, g/d											
	11	12	13	14	15	16	17	18	19	20	21	22
3 ppm Zn	17	19	18	17	15	16	12	15	12	6	3	3
40 ppm Zn	20	20	20	21	17	18	19	16	19	10	5	5
40 ppm Zn (Sham operated)	20	19	21	20	20	21	21	20	18	14	13	13

TABLE 2
Weight Gain and Litter Size

Diet	Rat	Body wt at conception, g	Body wt at parturition, g	Change in wt, g	Total pups in litter
3 ppm Zn, induced labor—d 22	A22	260	322	62	8
	A21	245	274	29	8
	A29	250	315	65	9
	A36	269	370	101	12
	A35	268	345	77	13
40 ppm, induced labor—d 22	A20	250	310	60	9
	A24	270	333	63	9
	A26	301	404	103	6
	A30	293	361	68	8
	A27	264	368	104	10
	A38	323	397	74	13
40 ppm, sham operation, spontaneous labor—d 23	A68	330	416	116	9
	A74	313	463	150	16
	A76	302	411	109	11
	A78	297	416	119	9
	A79	288	401	113	14

on d 17, but surgical stress also reduced food intake and weight gain to an extent that a pair-fed control was deemed unnecessary.

The rats in our colony normally deliver during the late afternoon of d 22 or the early morning hours of d 23. Oxytocin infusion beginning at 12 noon on d 22 was effective in inducing labor in all five low-zinc rats and in five of six control animals. Base-line measurements prior to infusion showed irregular uterine pressure cycles of low amplitude in both groups. As infusion commenced, the control rats displayed dose-dependent increases in both the frequency and amplitude of the pressure changes, yielding a regular pattern (eg., Figs. 1, A26, and 2, A38). In the one rat (A24) in which the oxytocin administration failed to induce labor, the pressure cycle pattern was nevertheless similar to that of the other controls. Among the low-zinc group, one rat (A35) displayed a pressure

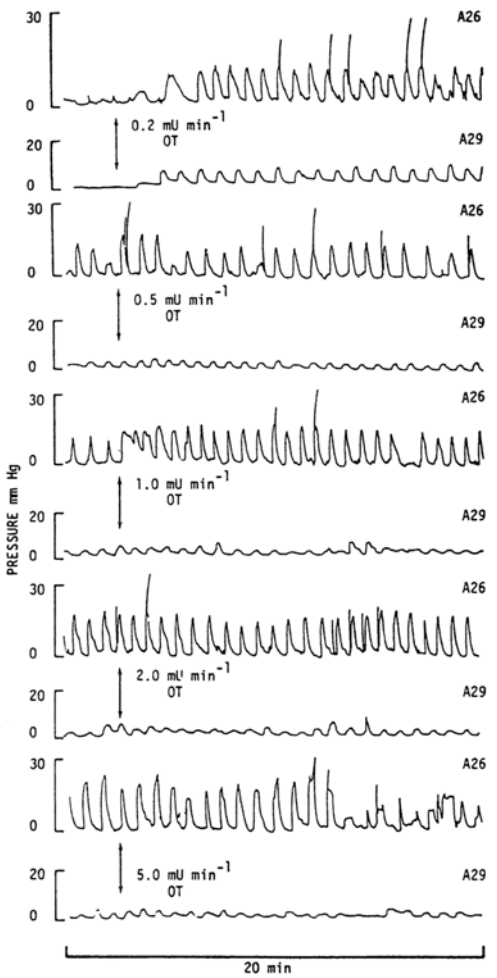

Fig. 1. Intrauterine pressure recordings in rats A26 (control diet) and A29 (3-ppm-zinc diet) as a function of oxytocin dose and time. A26 shows a normal pattern. The pattern for A29 is of very low amplitude.

cycle pattern identical to that of the control group and delivered 12 pups in 136 min (Table 3). Two low-zinc-fed females developed pressure cycles of very low amplitude (eg., Fig. 1, A29), whereas the remaining two rats had abnormal patterns (eg., Fig. 2, A36) consisting of sustained increases in basal pressure and only occasional low-amplitude pressure cycles.

The uterine pressure cycle characteristics were also altered at the time of birth by the low-zinc diet. In general, the birth of the first pup in either group was preceded by a period of intense abdominal straining, registered on the recording trace as spikes. Thereafter, the delivery of subsequent pups or placentae in zinc-adequate rats was usually synchronous with a single abdominal contraction (Fig. 2, A38), and the emergence time was less than 1 min. In the low-zinc group, fetal expulsion

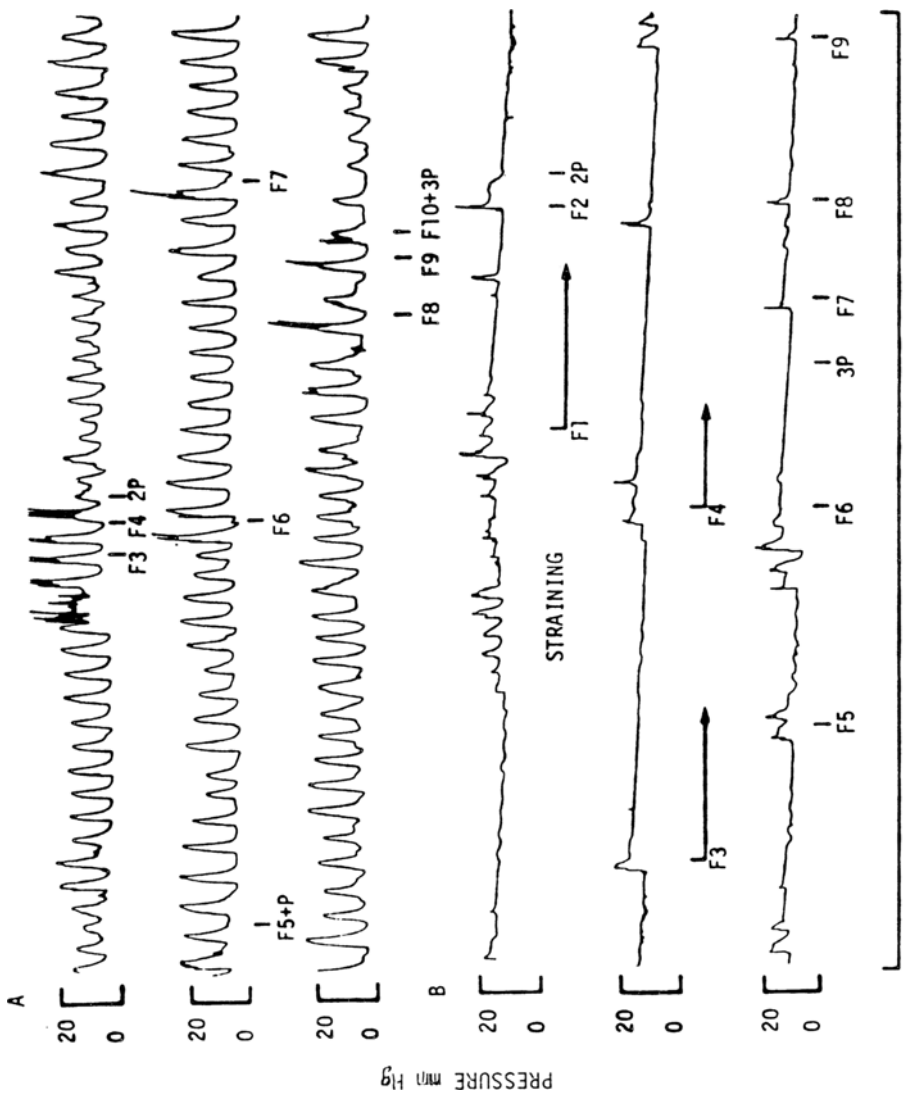

Fig. 2. Intrauterine pressure recordings in rats A38 (control diet) (A) and A36 (3-ppm-zinc diet) (B) during delivery. Spikes on the tracing are produced by abdominal straining. Delivery of fetuses and placentae are designated as F and P, respectively. Arrows depict emergence time.

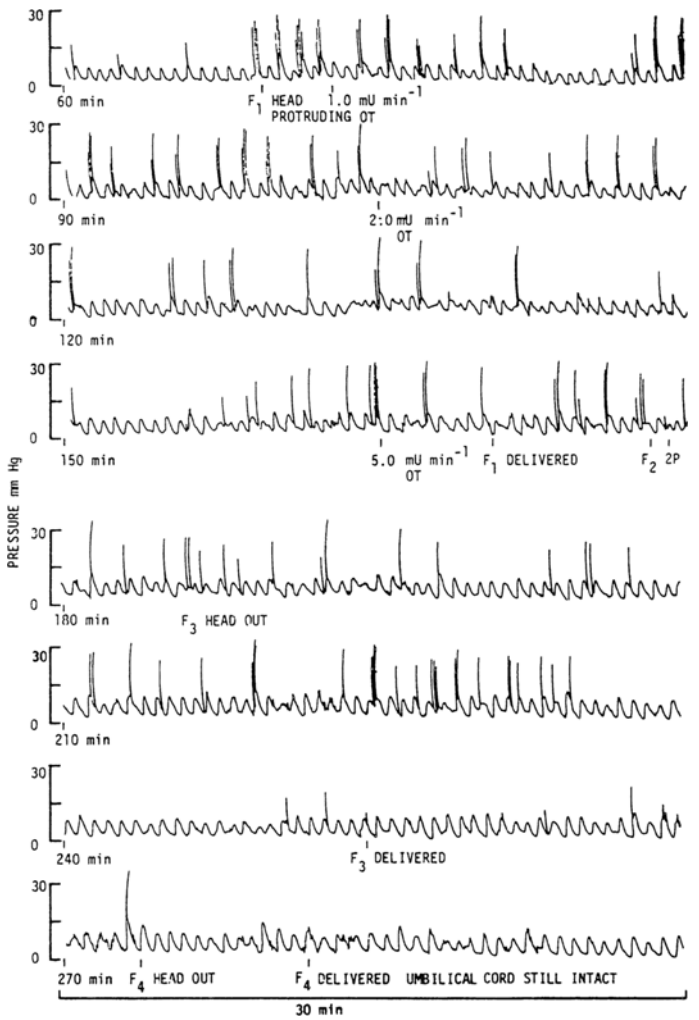

Fig. 3. Intrauterine pressure recording in rat A21 (3-ppm-zinc diet). Fetal expulsion time prolonged and accompanied by considerable abdominal straining seen as spikes on the tracing.

was usually prolonged to 1–6 min and, in one case (Fig. 3, A21), to over 100 min and was associated with prolonged periods of abdominal straining. Many pups were either stillborn or died shortly after birth. The zinc-deficient dams showed little interest in the birthing process, and it was not uncommon for the umbilical cord to remain intact and for one or more pups to be dragged about the cage until the placentae were delivered.

Results of the analysis of the plasma for zinc and progesterone and of the uterus for estrogen receptor content and affinity are shown in Table 3. In a previous study (*10*), plasma zinc was seen to decline gradually in control rats as they approached delivery (1.46 ± 0.07 μg Zn/mL on d

TABLE 3
Physical and Biochemical Data at Delivery

Diet	Rat	Plasma zinc, μg/mL	Proges-terone, ng/mL	No. pups born	Time from start of infusion to birth of first pup, *min*	Delivery time from 1st to last pup, *min*	Mean fetal wt, g	Estrogen receptor affinity constant, $K_a \times 10^{10}$ *M*	Estrogen receptor number, pmol/ uterus
3 ppm	A22	0.50	4.0	5	150	69	5.0	0.04	0.87
	A21	0.42	27.3	4	71	>290	3.6	0.08	0.14
	A29	0.65	4.8	1	247	>142	5.0	0.02	0.79
	A36	0.47	13.8	11	294	80	5.7	0.08	0.78
	A35	0.44	8.1	12	106	136	4.9	0.02	1.49
	$\bar{x}$ ± SEM	0.50 ± 0.04	11.6 ± 4.3				4.8 ± 0.3	0.05 ± 0.01	0.81 ± 0.21

40 ppm	A20	0.42	15.1	7	116	49	4.8	0.04	0.68
	A24	0.40	7.8	0	Not induced		6.0	0.01	0.96
	A26	0.73	10.0	5	137	73	5.4	0.03	1.56
	A30	0.47	13.3	8	76	89	4.3	0.04	1.33
	A27	0.69	14.9	10	205	100	5.6	0.05	1.06
	A38	0.56	6.9	10	94	200	5.6	0.04	1.25
	$\bar{x}$ ± SEM	0.54 ± 0.06	11.3 ± 1.5				5.3 ± 0.3	0.04 ± 0.01	1.14 ± 0.13
40 ppm, sham operation	A68	1.06	3.7	7	Day 23	NM[c]	6.3	0.04	0.95
	A74	0.82	4.0	16	Day 23	70	5.0	0.04	1.24
	A76	0.72	3.6	9	Day 23	49	6.7	0.04	1.21
	A78	0.72	5.4	9	Day 23	NM[c]	8.2	0.09	0.44
	A79	0.82	2.0	8	Day 23	>75	6.0	0.03	1.40
	$\bar{x}$ ± SEM	0.83 ± 0.06[a]	3.7 ± 0.05[b]				6.4 ± 0.5	0.05 ± 0.01[d]	1.05 ± 0.17

[a] $p < 0.002$, one-way ANOVA.
[b] $p < 0.01$, Kruskal–Wallis one-way ANOVA.
[c] NM = Not measured.
[d] $p < 0.03$, one-way ANOVA.

19 vs 1.02 ± 0.05 μg Zn/mL on d 23). Rats fed an acutely zinc-deficient diet (<1 ppm) showed a plasma zinc of 0.47 ± 0.02 μg/mL on d 19 and no appreciable change thereafter (0.44 ± 0.03 μg/mL on d 23). In the present study, at the conclusion of oxytocin-induced labor, plasma zinc was 0.50 ± 0.04 μg/mL in the rats fed the 3-ppm-zinc diet and 0.54 ± 0.06 μg/mL in the control rats. Plasma zinc in the sham-operated controls at the conclusion of delivery was 0.83 ± 0.06, which was significantly higher than the other two groups ($P < 0.002$). Plasma progesterone was also similar at d 22 in the low-zinc (11.6 ± 4.3 ng/mL) and zinc-supplemented controls (11.3 ± 1.5 ng/mL), and there was a further decline in the sham-operated controls killed after delivery on d 23 (3.7 ± 0.5, $P < 0.01$). There was no difference in uterine size as a function of treatment. Uterine estrogen receptor number was 0.81 ± 0.21 pmol/uterus in the rats fed the low-zinc diet, compared to 1.14 ± 0.12 in the 40-ppm-zinc controls and 1.05 ± 0.17 in the 40-ppm-zinc sham-operated group. The low-zinc group did not meet the 5% level of probability for a statistically significant difference in either estrogen receptor number or affinity constant. It should be noted, however, that the four zinc-deficient rats that showed the typical behavioral and physical disabilities of this condition had estrogen receptor numbers below five of the six controls, whereas the one low-zinc animal (A35) with a normal pressure cycle pattern was the only one to have an estrogen receptor value above 1 pmol/uterus.

DISCUSSION

The successful transition from gestation to labor and delivery requires a carefully regulated and harmonized series of events (*14*). Central to this transition is the removal of progesterone and the achievement of estrogen dominance. The initial decline in progesterone output begins in the rat at about 96 h prepartum. By approximately 24 h prepartum, a ratio of estrogen to progesterone is achieved that allows increased estrogen receptor synthesis and results in increased uterine sensitivity to estrogen. One of the major estrogen-dependent events is the appearance of numerous gap junctions in the uterine myometrium. During late pregnancy in the rodent, unstimulated uterine smooth muscle displays spontaneous, arhythmic, uncoordinated contractions. With approaching parturition, the frequency, amplitude, coordination, and propagation of contractile activity increases. This appears to be accomplished through the agency of gap junctions, which provide low resistance coupling for cellular communication (*15,16*). The pressure cycle alterations observed in the present study demonstrate a disruption in the coordination and propagation of uterine contractile impulses, which is probably responsible for the clinical impression of delayed, difficult, and prolonged parturition in zinc-deficiency rats. It is possible that zinc plays a direct role in

the contractile process. Daniel et al. (*17,18*) have reported that contractile responses of rat uteri suspended in a bathing medium and exposed to submaximal doses of acetylcholine are potentiated by zinc in concentrations of 10^{-6}–$10^{-4}M$. We belive it to be more likely, however, that the aberrant pressure cycle patterns stem from the approximately 50% deficit in number of myometrial gap junctions, which we have described in a separate paper (*19*).

These results suggest, therefore, that zinc may be necessary for the timely uptake and processing of estrogen and possibly other steroid hormones. Such a conclusion finds support in previous in vitro studies. Shyamala and Yeh (*20*) concluded that the mouse mammary-gland estrogen receptor is a zinc metalloprotein, and Lohmar and Toft (*21*) came to a similar conclusion with regard to the chick oviduct progesterone receptor. Colvard and Wilson (*22*) found that physiological concentrations of zinc ion potentiated binding of androgen receptor to isolated rat prostate tumor nuclei. Moreover, there are numerous enzymes, such as DNA (*23*) and RNA polymerases (*24*), reverse transcriptase (*25*), restriction enzyme *Eco*RI (*26*), and SI nuclease (*27*), which require zinc in order to bind in tight association with specific DNA sequences. It may also be relevant that zinc deficiency in human male adolescents was associated with arrested gonadal maturation and that zinc supplementation in such individuals yielded spectacular recovery (*28*).

The significantly lower plasma zinc in the oxytocin-induced, zinc-adequate controls, relative to the spontaneously delivered, sham-operated controls, deserves some comment. We presume that it occurred as a response to the d-19 surgery. Lindeman et al. (*29*) have reported a rapid fall in plasma zinc after acute tissue injury associated with surgical procedures. The sham operation was milder than that required for measurement of the pressure cycle, hence, it would have a lesser effect on plasma zinc. The sham-operated animals also had an additional day to recover. It is also possible that the oxytocin infusion promoted a decline in plasma zinc, although this has not been previously reported. Perhaps, more interesting, is the recognition that, despite equivalent plasma zinc values, the rats previously maintained on an adequate dietary zinc intake displayed normal pressure cycle patterns and delivery, whereas those fed the low-zinc diet experienced difficulty. Thus, the plasma zinc level *per se* may not be as important as the pool of zinc available for transfer to or within cells. Plasma zinc may nevertheless be informative. Durá–Travé et al. (*30*) measured plasma zinc in 208 pregnant women in Spain during gestation and at the moment of delivery. They found a significant ($P < 0.05$) inverse correlation between plasma zinc (83 ± 21 μg/dL vs 65 ± 17 μg/dL) and duration of delivery (<5 h vs >10 h). In 10 cases requiring a cesarean section because of uterine atony, the plasma zinc was 63.4 ± 12.4 μg/dL. The relationship between zinc status and uterine contractile competence at delivery observed in rats should be further explored in humans.

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