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ZINC AND REPRODUCTION[1]

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CONTENTS

The severe effects of Zn deficiency on reproduction in animals, both male and female, have been known for many years (183). The observations were not considered to have much relevance to human health, however, because the occurrence of Zn deficiency in humans was deemed unlikely. Attention was drawn to the importance of Zn in human health both by the dramatic response of the congenital disease acrodermatitis enteropathica to Zn supplementation (183) and by numerous reports of Zn deficiency occurring in conjunction with total parenteral nutrition. The occurrence of Zn deficiency under clinical conditions indicated that it was not as rare a condition as had been thought, and the question of whether it might be involved in reproductive problems in humans assumed more importance. Zn deficiency was of particular concern for women because their Zn intake is generally less than the recommended daily allowance (RDA) for adults and is substantially lower than the RDA of Zn

during pregnancy. Determining whether Zn deficiency is involved in human reproductive problems, however, is hampered by lack of a sensitive indicator of Zn status. Nonetheless, because of the importance of the question, numerous studies have been done both in humans and animals to determine the extent to which Zn deficiency may be related to reproductive problems and to elucidate the role of Zn in reproductive function. This review is limited primarily to work published in the last ten years. Much of it, of course, confirmed and refined earlier observations; for reviews of earlier work the reader is directed to References (45, 99, 183).

FEMALE

Zn Status and Outcome of Pregnancy

Zn deficiency in the pregnant rat had been known for some time to result in malformations and in difficult delivery (183), but Jameson (101) in 1976 was the first to report large-scale studies to determine whether pregnancy complications in humans were related to inadequate Zn status. Although serum (or plasma–in this discussion we do not distinguish between them) Zn concentration is not a sensitive indicator of Zn status, it is widely used because it is easy to measure and because a better indicator has not been established. Jameson therefore measured Zn concentrations in weeks 9–23 and related them to outcome of pregnancy. Abnormalities either in delivery or in the infant were associated with lower concentrations, 90 μg/dl, compared to 104 μg/dl for normal deliveries. A further study in which a special effort was made to find women with either a high risk or a past history of complications produced similar results. Women with normal deliveries and infants had higher Zn concentrations during weeks 20–22 than women who had abnormal deliveries or infants, 94 vs 86 μg/dl. Eight women with malformed infants had a mean Zn concentration of 80 μg/dl. Although the differences were small, the suggestion that inadequate Zn status was associated with malformations and abnormal deliveries prompted a number of studies.

MOTHER Development of pregnancy hypertension was associated with serum concentrations 10–14% lower than the mean earlier in pregnancy (51, 192). Zn supplementation reduced the occurrence of pregnancy-induced hypertension in Mexican-American women, but the occurrence of hypertension was not related to serum Zn concentrations (97). Serum Zn concentrations were 20–30% lower in severe preeclampsia than in controls (25, 164); infant concentrations were also reduced, but the maternal/fetal ratio was the same as in normal pregnancy (25).

Serum concentrations were lower in women with increased length of labor in one study (65) but not in another (51). Serum Zn was lower in ten women

delivered by cesarean section because of "uterine atony" (65). Uterine atony was associated with low serum Zn together with low hemoglobin concentration in one of Jameson's initial studies (101). On the other hand, decreased Zn was observed in women with "enhanced uterine muscle irritability" (35). Women delivering preterm infants had lower serum concentrations, but there was no correlation with premature rupture of membranes (104). Women with prolonged pregnancy (≥42 weeks) also had low serum Zn (9). Serum Zn in the lowest quartile of Zn concentration was associated with pregnancy complications (134). Others found no relation between serum Zn and complications of pregnancy (2, 8, 100, 149, 167). Serum Zn concentrations were lower than one standard deviation below the mean in 7 of 25 first-trimester abortions (40), not different from the mean in six second-trimester abortions (41), and below the mean in two others (186).

Results in animals were also variable. Serum Zn in the cow decreased during labor with a further decline with dystocia in one report (64) but not in another (152). There was no difference in serum Zn in the ewe at parturition with or without dystocia (127).

INFANT Malformations have been associated with low maternal serum Zn concentrations (41, 48, 51, 70, 149, 169), with elevated cord serum Zn (192), and with higher than normal ratios of maternal/fetal serum Zn (70). A slight increase in hair Zn concentrations from distal to proximal end was observed in mothers of spina bifida infants in contrast to a slight decrease in mothers of normal infants. Infant hair Zn did not differ (31).

Small-for-gestational-age infants did not have serum concentrations different from those of larger infants; maternal serums also were not different (36, 75, 129, 163, 184). Leukocyte Zn concentration of infants with prolonged intrauterine growth retardation was significantly reduced, but infants with acute intrauterine growth retardation and preterm infants did not differ from normal infants (129). Preterm, full-term but low-birth-weight, and normal infants did not differ in hair Zn concentration (74). A tendency toward negative correlations between maternal serum Zn and infant birthweights was reported in several studies (4, 51, 61, 132, 149); in one study a correlation was apparent in the second but not third trimester (134).

AMNIOTIC FLUID There have been a few attempts to relate Zn concentration in amniotic fluid to outcome of pregnancy. Women with hypotrophic fetuses (112), diabetes (112, 141), gestosis (112), or prolonged pregnancy (9) had lower concentrations in some studies but not others (159, 168, 184). Threatened asphyxia of the fetus resulted in lower Zn concentrations in one study (193) but higher values due to meconium in another (191).

The heterogeneity of amniotic fluid and the high Zn concentration in meco-

nium (7, 112, 159, 191) complicated Zn analysis. Half of the amniotic Zn was associated with particulates that sedimented during centrifugation. Meconium contamination was identified by measurement of absorption at 405 nm (159). Mean Zn concentrations were 5–15 μg/dl in 10 of 18 studies reviewed; the range was 5–35. Concentrations changed little with gestational age (30, 52, 159) except in two studies (38, 112) in which it increased 2–4 weeks before parturition. The concentration in monkeys was similar to that in humans (78), whereas the concentration was higher in rats (91, 106) and still higher in sows (107). Zn deficiency lowered concentrations by a third in monkeys (78) and rats (91).

ZINC SUPPLEMENTATION Another and more difficult approach to determining whether Zn status is related to outcome of pregnancy is to supplement women suspected of having Zn inadequacy and look for improved pregnancy outcome. Low-income Mexican-American women supplemented with 20 mg of Zn daily had a lower incidence of pregnancy-induced hypertension than did unsupplemented women; no other difference in complications was associated with supplementation (97). Supplementation of low-income women in India with a much larger amount of Zn, 300 mg $ZnSO_4$ daily, was stopped after three premature births and one stillbirth occurred consecutively (138). Supplementation of a small number of presumably well-nourished women with a large amount of Zn (90 mg) had no deleterious effects, however (101); so the effect of Zn, perhaps on absorption of other nutrients, was more critical in less well-nourished subjects, if in fact the unfavorable outcome was due to Zn toxicity.

To test for deleterious effects of Zn on outcome of pregnancy, rats were given a 10% protein diet and 150 ppm Zn from day 1 of pregnancy. The number of fetuses per female on day 18 was the same, 8.0 and 8.2, for experimental and control rats respectively. The number of implantations was larger in the high-Zn group, however, 8.9 compared to 8.4 (138). The larger number of resorptions in the high-Zn group was attributed to high Zn intake; but in our experience, in normal rats the larger the number of implantations, the greater the resorptions. Rats are probably not a good model for testing the toxicity of Zn during pregnancy since they can tolerate quite high levels of Zn. Feeding 1000 ppm throughout pregnancy, for example, did not increase the number of resorptions or stillbirths (156) nor did injections of 1 mg daily from days 6–20 (63).

Since women with low serum Zn concentrations are another group that may have inadequate Zn status, Jameson investigated the effect of Zn supplementation on women in this category. Seven of 20 women with low serum Zn concentrations and unsatisfactory hemoglobin concentrations in spite of oral Fe and vitamin supplementation were supplemented with Zn, 90 mg daily, during

a 10–12 day hospital stay. They presumably continued to take the supplements for the remainder of gestation (5–15 weeks). At delivery these women had shorter labors and less blood loss than the 13 unsupplemented women, six of whom experienced "severe hemorrhage with uterine atony" (101).

In a further study half of the women with serum Zn concentrations less than the mean, 65 μg/dl, on week 14 of pregnancy were given 45 mg of Zn daily. Of 69 unsupplemented women, 33 had normal deliveries compared to 40 of 64 supplemented women. (Compliance by the supplemented women was apparently assumed.) Normal deliveries and infants resulted from only 26% of the pregnancies in which serum Zn at week 14 was less than the mean and declined thereafter (102).

Serum Zn Concentrations

MATERNAL Although serum Zn concentrations were known to be reduced in late pregnancy (183), Jameson's results raised the question of how much of a reduction could be assumed to be physiological. Review of a few of the numerous reports now in the literature indicated general agreement that the values in late pregnancy were less than nonpregnant values, but there was less agreement on the magnitude of the decrease. In 19 studies with values for nonpregnant women as well as those in late pregnancy, pregnancy values were 10–30% lower in half the studies and 30–50% lower in the others. Fifteen of 22 studies reported mean values of 50–75 μg/dl for women from week 36 of gestation to term, with a range of 43–115 μg. Mean concentration for nonpregnant women in 12 of 21 studies was 78–95 μg/dl, with a range of 67–160. There were fewer data to determine the shape of the curve between the beginning of pregnancy and the end. In three studies (96, 98, 101) concentrations decreased during the first two trimesters with no change thereafter; in others the decline continued for a longer period (38, 87, 134, 151). The decline occurred too early to be due to increased plasma volume or to increased needs of the fetus. Since it occurred about the time that Cu concentration increased, it may have been controlled by the same mechanism (87). Tentative standards for the lower limit of serum Zn concentrations during pregnancy have been proposed, with a minimum of 40 μg/dl at term (87). In two recent studies 25% of the women had values below 40 μg/dl, however, with normal pregnancy outcomes (4, 43).

Maternal serum Zn also decreased at the end of pregnancy in the rat (42, 83), cow (64, 152), ewe (123), sow (142), and monkey (78). Binding of Zn by human pregnancy serum was reported to be slightly reduced in one study (76) but not different from nonpregnant serum in another (173).

Zn supplementation has also been used to investigate whether low serum Zn concentrations during pregnancy are related to inadequate Zn status. Supplements of 15 to 25 mg per day did not increase serum Zn concentrations (87, 96,

172). Concentrations at the end of a week were higher in the hospitalized women in Jameson's study, but a much larger Zn supplement was given (101). Serum Zn concentrations responded rapidly to changes in Zn intake in the ewe (17, 67, 126), monkey (175), and rat (183).

FETAL Zn concentration of cord blood serum was higher than that of maternal serum in all the studies reviewed except in an acrodermatitis enteropathica pregnancy in which maternal and cord values were the same (39). The magnitude of the difference varied; 12 of 18 studies reported values 110–150% and the rest 150–200% of maternal serum. Fetal plasma Zn concentrations were approximately 200% of maternal concentrations in the monkey (79) and ewe (16, 126) and were reduced by low Zn intakes of the dam, but the fetal/maternal ratio was not changed. Increased concentration of Zn in human cord serum was not due to increased protein since total protein, albumen, and $\alpha_2$macroglobulin did not differ in maternal and cord serum (44, 192).

NONPREGNANT In women, serum Zn concentration rose during the menstrual cycle and peaked at midcycle; no peak occurred in women with anovulatory cycles (85, 144). In estrus-synchronized ewes serum Zn was higher at estrus and metestrus in the first cycle but not the second (92). Zn losses of 0.2–0.5 mg during the menstrual cycle have been reported (82, 90, 182).

Although oral contraceptives were reported to lower serum Zn concentrations (183), three studies found no difference between users and nonusers (90, 94, 185) and one study reported a decrease within 24 hours with no further change (150). Hair Zn concentration did not differ between users and nonusers (185).

Other Indicators of Zn Status

Measurements of other suggested indicators of Zn status have also been made in pregnant women and newborns. As noted above, leukocyte Zn concentration of infants with prolonged intrauterine growth retardation was significantly reduced. Since serum Zn concentrations were not different, leukocyte Zn was considered to be a better indicator of Zn status (129). Maternal leukocyte Zn as well as serum Zn decreased during pregnancy (130). Leukocyte Zn concentrations in ewes did not differ with duration of pregnancy or with Zn intake (67).

Small decreases in hair Zn concentration of pregnant compared to nonpregnant women were reported (31, 96, 186) although in one case the decrease was significant only for pubic hair (57). Other investigators found no difference between pregnant and nonpregnant samples (87, 105, 172, 174, 181). At the end of pregnancy, hair Zn was higher in Zn-supplemented than in Zn-unsupplemented women in one study (87) but not in another (96). Mean concentrations of Zn in infant hair were slightly higher than in maternal hair,

180–240 μg/g compared to 160–200 μg/g. Lower concentrations were found in pubic than in scalp hair (57, 141).

Salivary Zn concentrations did not change during pregnancy and ranged from 30 to 70 ng/ml (87, 172, 174). Women supplemented with Zn during pregnancy had higher concentrations than unsupplemented women in one study (87) but not in others (172, 174). Salivary Zn concentration in pregnant rats was higher than in humans and was not changed by Zn deficiency (66).

Urinary Zn excretion was lower in pregnant than nonpregnant women during the first trimester (87, 88). Excretion increased as pregnancy progressed (88) but was not always significantly different from nonpregnant values (87, 105, 172, 174, 181). Less than 100–150 μg urinary Zn excretion per day was proposed as suggestive of inadequate Zn status (181). Urinary Zn excretion did not differ between pregnant and nonpregnant sows (107).

Alkaline phosphatase was consistently higher in Zn-supplemented pregnant women than in those unsupplemented (87). In Mexican-American women, those with serum Zn lower than one standard deviation below the mean had elevated serum RNAse and decreased red blood cell ALA-dehydratase (97).

Dietary Zn

In eight of ten studies reviewed, daily Zn intake by pregnant women calculated from dietary recalls was remarkably similar, 9–11 mg, whether the women were Lebanese (181), Mexican-American (96, 98), vegetarians (4), middle-income Americans (40, 87, 133), or Britons (3). Two studies reported slightly higher intakes, 12–14 mg (43, 105), and one reported 7 mg per day for Asian vegetarians (3).

The RDA for Zn during pregnancy is 20 mg (137), almost double the estimated intake of most pregnant women. The RDA was based partially on an assumption of reduced Zn absorption during pregnancy. Pregnant and nonpregnant women absorb and retain Zn equally, however (172, 174). Reduced absorption of Zn was also thought to occur in vegetarian women since Zn from plants has been considered less available than that from animal sources, but serum Zn concentrations of pregnant vegetarian women were generally not different from nonvegetarians (4, 105, 192). Zn absorption in rats increased in late pregnancy (58, 166). Sows given a high Zn intake absorbed more Zn than nonpregnant animals during the first two trimesters but not the third (108); with a lower Zn intake both pregnant and nonpregnant sows were in negative balance to the same extent (107, 108).

Concern has been expressed that large Fe supplements sometimes taken during pregnancy may depress Zn absorption. Serum Zn concentrations were lower in women taking Fe supplements in three studies (40, 87, 128) but not different in another (4). Serum Zn concentrations during a Zn tolerance test were reduced in ten women who were supplemented for two weeks with 100

mg ferrous fumarate and 5 mg folic acid. The results were consistent with either decreased Zn absorption or redistribution of Zn to the tissues (128). Supplementing pregnant rats with four times the recommended amount of Fe had no detrimental effects in animals receiving a marginal Zn intake (69).

Zn Deficiency

MALFORMATIONS The deleterious effects of inadequate Zn status during pregnancy were demonstrated very clearly in animals fed diets deficient only in Zn. Zn deficiency in such a pure, or severe, form is, of course, unlikely in humans. The closest thing to it is the Zn-responsive congenital disease acrodermatitis enteropathica. Before the efficacy of Zn in treating this disease was known, three of seven pregnancies in women with the disease resulted in malformed infants or stillbirths. With adequate Zn supplementation two such pregnancies had normal outcomes (39).

Zn deficiency also caused malformations in the chick and rat (183). Since the extensive work with the rat was recently reviewed (99), we do not discuss it here. Malformations due to Zn deficiency have been reported in species other than the rat and chick but to a much lesser extent. "Abnormal" pigs were produced by sows with a moderate Zn intake (93); abnormal ossification of fetal bone occurred in pigs with a low Zn intake (142), and malformed lambs and mummified fetuses were produced by ewes (17). No malformations were seen in monkeys (79, 175).

DYSTOCIA Zn deficiency during pregnancy in the rat also resulted in severe stress of the female at parturition. Prolonged labor, excessive bleeding, failure to eat the afterbirths or care for the pups, and sometimes death at parturition were among the abnormalities observed (11–13, 135, 140). Body temperature, blood pressure, and serum corticosterone were decreased 3–6 h post partum (140, 155). In our experience malformations and stress did not occur in the same experiment. In experiments in which malformations occurred, litter size was reduced and the females were not stressed. What determined whether malformations or stress occurred was never clear. The degree of stress was related to litter size; females with fewer than ten pups were usually not stressed (11, 13). Small litters also reduced the severity of the effect on the fetus (157).

DECREASED FEED INTAKE Part of the stress in the rat was undoubtedly due to the drastically reduced feed intake. The effect on food intake was most severe during the latter part of pregnancy when deficient rats ate almost nothing (12); feed intake increased with increasing dietary Zn (71).

Use of a "pair-fed" control is a time-honored way to try to distinguish between the effects of low food intake and the lack of a specific nutrient.

Besides differences introduced by "meal-eating" by pair-fed animals, Zn deficiency adds another variable, cyclical feed intake (183), particularly in young rats. Since cycling is not synchronous, pair-fed rats that are fed the average intake of Zn-deficient rats will have a fairly constant daily feed intake instead of the fluctuating intake of Zn-deficient rats. Pregnant rats in our experiments and in others (71, 80) did not feed cyclically; other investigators have observed it, however (42, 154).

Since pair-fed controls utilize feed more efficiently, they usually gain more weight than deficient animals. With young growing animals this results in comparisons between animals that are at different stages of development. The difference this can make was evident in comparisons of reproductive hormone concentrations in young male rats; pair-weight controls differed less from protein-deficient animals than did pair-fed controls (77). Another difference that is introduced by some investigators is use of EDTA-extracted protein and additional phytate in the diet for the Zn-deficient group only.

With pregnant rats not only is weight of the female greater in pair-fed controls but also weight of the fetuses. Parameters measured in Zn-deficient and pair-fed fetuses on the same day of gestation may therefore be comparing different stages of development due to different growth rates in the fetuses. Furthermore, even if the female's weight gain is restricted to that of the experimental rat, the Zn-adequate rat may still supply more nutrients to the fetuses than the Zn-deficient animal. In interpreting results therefore it is necessary to keep in mind the many other factors in addition to Zn intake that can contribute to differences observed.

In this discussion we do not distinguish within groups but refer to all types of restricted feed intake controls as "+ZnRI," to ad-libitum-fed controls as "+Zn," and to Zn-deficient animals as "−Zn."

Force-feeding to compensate for reduced feed intake was also attempted but was unsuccessful because of stress to the animal (11, 124). Stress was as severe in +ZnRI as in −Zn rats and may have been related to the viscosity of the solutions (11). Feed intake was much more severely affected in rats than in monkeys (78) or ewes (17, 126).

Reduced feed intake resulted in tissue catabolism, which released Zn; Zn deposited in the fetuses exceeded the female's Zn intake by 200–300% (124, 125). Fewer malformations and resorptions occurred in −Zn females with additional feed restriction from the beginning of pregnancy, presumably because of Zn made available by additional tissue catabolism (124). Variation in the timing of feed intake cycles with consequent release of tissue Zn due to catabolism has been proposed to explain the variation in occurrence of malformations in rats (154). Since cycling did not always occur in pregnant rats, other factors must be involved. Although very low Zn intakes increased tissue catabolism, marginal Zn intake did not alter urinary nitrogen excretion (80)

or serum glucose or amino acids but did increase β-hydroxybutyrate oxidation (81), probably as a result of reduced feed intake.

PARTURITION The mechanism(s) responsible for the marked stress of −Zn rats at delivery has not been determined. Stress as severe as that in the rat has not been observed in other species although farrowing was prolonged in −Zn sows (142), and −Zn ewes with normal-sized lambs tended to have more difficulty than +Zn ewes (17, 18, 122). There were greater losses and more complications in +ZnRI and −Zn monkeys, but no difference in incidence between the groups (79). Prolonged gestation was observed in the −Zn sow (142) and rat (42, 135) but not in the monkey (79) or ewe (17, 122, 126). Excessive blood loss such as that observed in −Zn rats at parturition (11, 55, 140) has not been reported in other species except in the pig in which the cord was abnormally porous (142).

Among the things studied in connection with dystocia in the rat were hormones, both reproductive and stress-related, prostaglandins, uterine contractility, hemodynamic adjustments, and factors related to blood clotting. To take the last first, bleeding in the rat was not due to reduced platelet aggregation (189) nor to prolonged prothrombin clotting times (J. Apgar, unpublished). Bleeding may have resulted from failure of blood vessels to contract properly (140).

Relatively few measurements of hormone and prostaglandin concentrations have been made in −Zn animals. Comparisons are difficult in the rat because of the rapidity of the changes at the end of pregnancy and the difficulty of taking serial samples without stressing the rat, especially the −Zn rat in which blood volume may be reduced (11). In addition to the reduction in feed intake, which is known to affect many hormones and prostaglandins, the prolonged gestation of the −Zn rat further complicates comparisons because the −Zn rat on day 22, for example, will not be at the same stage relative to delivery that the +Zn rat is.

Serum progesterone concentrations were higher in −Zn and +ZnRI than in +Zn rats on day 18 (11), and on day 23 were still higher in −Zn rats than in the controls (42). Prolonged gestation was attributed to the higher ratio of progesterone to estrogen in −Zn than +Zn rats at the end of gestation; serum estrogen concentrations were not different (42). Serum progesterone and prolactin were lower in −Zn than +Zn ewes prior to parturition, but peak prolactin concentrations at lambing were not different (J. A. Fitzgerald, unpublished).

Serum corticosterone was lower in −Zn rats 3–6 h post partum (155). Neither injections of aldosterone (140) nor corticosterone (J. Apgar, unpublished) during the latter part of pregnancy alleviated the stress of −Zn rats, although corticosterone reduced adrenal hypertrophy and increased adrenal cholesterol.

Because prolonged gestation and difficult delivery resulted from aspirin administration to +Zn rats, stress in −Zn rats was attributed to reduced prostaglandin production (140). Delayed appearance in −Zn rats of an ovarian enzyme related to delivery and considered to be induced by $PGF_{2\alpha}$ supported the hypothesis that Zn deficiency interfered with prostaglandin synthesis (42). Measurements of prostaglandins or their metabolites, however, indicated that prostaglandin concentrations were increased rather than decreased in pregnant −Zn rats. Where concentrations were increased in tissues of −Zn rats, they were similarly increased in the tissues though not serum or urine of +ZnRI controls (68, 139). Pharmacological doses of $PGF_{2\alpha}$ shortened gestation in −Zn as well as +Zn rats (42). The −Zn rat was therefore able to respond to a strong stimulus, at least if it was applied before the rat was excessively weakened by failure to eat.

Recovery in various prostaglandins of radioactivity from labelled arachidonic acid added to uteri or placenta in vitro differed between −Zn and +Zn rats (56); but whether the pool size of arachidonic acid also differed between the two groups was not determined and would have affected the conversion rate observed. Serum PGFM was lower in −Zn than +Zn ewes at parturition but was probably related to reduced viability of −Zn fetuses (J. A. Fitzgerald, unpublished).

Fewer high-amplitude uterine contractions occurred in vitro in −Zn than in +Zn rats (56); the number of observations was very small, however, and the difference could have been related to differences between animals in their status relative to time of delivery. Limited observations of uterine contractions in vivo in −Zn and +Zn ewes did not indicate any difference (16).

Failure to make hemodynamic adjustments during pregnancy has been cited as the cause of fetal growth retardation in feed-restricted rats. Cardiac output was reduced by 30% in rats that were deprived of food during the last week of gestation; blood flow to the uterus and placenta was reduced by an equivalent amount (5). A 50% reduction in cardiac output with a 75% reduction in blood flow to the uterus and placenta was reported in rats deprived of Zn for the last week of gestation (56). The greater change in the Zn-deficient rat may have been due to the use of second-parity rats that had heavier body weights and a different pattern of feed consumption than did primiparous rats (71).

Failure to make hemodynamic adjustments may also have been related to stress at parturition in −Zn rats. Hematocrit, which decreased during pregnancy in the +Zn rat, markedly increased at the end of pregnancy in the −Zn rat (11, 13). A single injection of Zn on day 18 was followed by a prompt decrease in the hematocrit of −Zn rats and essentially normal parturition (12). The changes in hematocrit were presumably related to changes in plasma volume and suggested that plasma volume was decreasing in −Zn rats prior to parturition. Decreased serum protein concentrations during pregnancy in +Zn but not

−Zn ewes may also have been related to abnormal blood volume adjustments (J. A. Fitzgerald, unpublished). Although hematocrit increased in −Zn rats, serum protein was not different from that of +Zn animals (13, 15).

Other abnormalities observed in pregnant −Zn animals included a high incidence of abnormal red cell morphology during the third trimester of pregnancy in −Zn monkeys (78), and reduced spleen (13) and thymus (140) weights in rats.

VITAMIN A Stress at delivery in −Zn rats was very similar to that in vitamin A–deficient rats (120). If Zn were required for vitamin A metabolism as has been suggested (183), stress could be due to vitamin A deficiency. Fetal liver from −Zn rats given no vitamin A during pregnancy contained as high a vitamin A concentration as did +Zn livers, however; and −Zn rats given a diet without vitamin A for extended periods never developed the constantly cornified vaginal smears characteristic of vitamin A deficiency (14). Zn deficiency therefore did not affect the ability of the rats to use their vitamin A stores. In the monkey, serum vitamin A concentration was reduced to the same extent by Zn deficiency and restricted feed intake; serum retinol binding protein was not affected (24).

FETUS Low birthweights and poor survival, partly due to maternal neglect (12, 55, 60, 135), were consistently observed in −Zn rats. Zn deficiency during pregnancy reduced birthweight in mice (28), in lambs in some experiments (16, 17, 126) but not others (18, 122), and in male but not female monkeys (79). Poor survival would be expected in animals with low birthweights, but survival was also poor in pigs in which birthweights were not affected (142). Survival was reduced in mice and in lambs from ewes given a low Zn intake throughout pregnancy (17). Large numbers of resorptions were associated with malformations in −Zn rats (99); abortion or failure to maintain pregnancy also occurred in −Zn ewes (17).

Low Zn intake during pregnancy was consistently reflected in low Zn concentration of fetal livers in all species studied (16, 122, 126, 142, 156). Fe concentration was increased in fetuses from rats with very low Zn intakes (156) but not from those with marginal Zn intake (69). Pancreatic enzyme activities and glucagon concentration were reduced in the −Zn fetal rat; insulin concentrations were reduced in the +ZnRI as well as the −Zn fetus (157, 158).

Incorporation of ^{3}H-thymidine into DNA of −Zn fetal brain was decreased in late gestation (62). Since the incorporation of ^{3}H-thymidine into DNA is dependent on the activity of thymidine kinase, the reduction in radioactivity in DNA was probably due to the observed reduction in thymidine kinase activity in −Zn fetuses (153).

Norepinephrine and dopamine in fetal rat brain were not affected by marginal Zn intake (86). Behavioral differences were observed in offspring of both −Zn rats (145, 146) and monkeys (165). Both +ZnRI and −Zn rats were affected; infants of +ZnRI monkeys did not survive.

Offspring of mice given a marginal Zn intake during pregnancy had immunological abnormalities (28), some of which were reported to persist in second- and third-generation mice (27, 29).

NONPREGNANT ANIMALS Reproductive function in nonpregnant animals was also affected by Zn intake. Estrus cycles ceased in both monkeys (175) and rats (14) given low Zn diets for extended periods. Zn supplementation started before mating was more effective in increasing the lamb crop than supplementing during pregnancy alone (123).

Summary

Work associating unfavorable outcomes of pregnancy with poor Zn status in women prompted a number of studies of the relation between Zn status, determined primarily by maternal serum Zn concentration, and complications of pregnancy. As might be expected, especially in view of the insensitivity of current measures of Zn status and the relatively low incidence of pregnancy complications, some studies found a relationship and others did not. The possibility that some pregnancy complications may be related to poor Zn status emphasizes the importance of being able to identify those at risk. This is particularly difficult in pregnant women because, although there was general agreement that serum Zn, the most widely used criterion of Zn status, decreased during pregnancy, the extent of the decrease varied. Zn supplementation generally did not increase serum Zn in pregnant women although most dietary intakes were approximately half the RDA for pregnancy. Some decrease in serum Zn therefore is physiological; the question is, how much. Tentative standards have been proposed that should help to resolve the problem. Another problem to be resolved is whether the large Fe supplements taken by some pregnant women are detrimental to their Zn status; some investigators observed lower serum Zn concentrations in women taking these supplements.

Zn deficiency during pregnancy has produced malformations not only in the chick and rat but also in the lamb; less severe abnormalities were produced in the pig. Prolonged gestation and difficult delivery were observed in the rat and pig but not in the ewe or monkey. Dystocia in the rat was partly due to severely reduced feed intake; an abnormal progesterone/estrogen ratio and abnormal hemodynamic adjustments at the end of gestation were also implicated. Decreased prostaglandin production or interference with vitamin A metabolism did not appear to be responsible for difficulty at parturition. Zn deficiency

resulted in low birthweights and/or poor survival of offspring in all species studied.

MALE

The high concentration of Zn in the prostate and in seminal fluid and the hypogonadism observed in Zn-deficient males continue to spark interest in the role of Zn in male reproductive physiology. This section covers primarily studies of Zn concentrations and function in normal tissue and studies of induced Zn deficiency; it does not cover investigations of the relation of Zn to infertility and impotence in conditions such as uremia, cirrhosis, hemodialysis, and sickle cell anemia. In vitro studies of metal- and hormone-binding by prostatic tissue are also not included.

Zn Concentrations

SEMINAL AND PROSTATIC FLUID Mean Zn concentration in human seminal fluid was 13 to 17 mg/dl in six of eight papers reviewed (72, 95, 118, 119, 136, 143, 147, 190), with a range of 12–24. Less than 8 mg/dl was considered abnormal (109). Zn concentration was highly correlated with Ca and Mg (95). In split ejaculates the first fraction had a higher Zn concentration than the second, 24 compared to 8 mg/dl; expressed prostatic fluid had an even higher Zn concentration, 40 mg/dl (95, 118). Mean Zn concentrations in seminal fluid from animals were generally lower than that in humans: 3 mg/dl in bulls, 4–20 in boars, and 6 in dogs (21, 37, 188).

SPERM The concentration of Zn in human and rat sperm was similar, 6–12 $\mu g/10^8$ sperm (21, 170, 187); that in boar and bull sperm was lower, 1–2 μg (21). Most of the Zn was loosely bound since washing sperm with buffered salt solution removed 75% of Zn from bull and 50% from boar sperm. Little Zn was removed from human sperm unless albumen was present in the buffer solution, in which case 70–80% of the Zn was removed (21). In rat sperm Zn was concentrated in dense fibers of the tail in a protein fraction similar to keratin (46).

PROSTATE Prostatic Zn concentration was much lower in young boys, 120 $\mu g/g$ dry weight, than in adults. Men 20–30 years old had 650 $\mu g/g$; those over 30, 970 $\mu g/g$ (115, 117) although lower values were also reported (84). Concentrations of Zn within the prostate varied, particularly in the rat in which concentrations in the lateral lobe were higher than those in the dorsal and ventral lobes (73, 177). Smaller differences were found between the less

well-defined lobes of the human (179). Rhesus monkeys had 16 times as much Zn in the caudal as in the cranial lobe of the prostate (26). The highest concentration of Zn in subcellular organelles of the prostate was in the nuclear fraction (113), particularly the nucleoli (26).

In human prostatic fluid and the first fraction of split ejaculates (which contained most of the prostatic secretion) most of the Zn was bound to a low-molecular-weight fraction (22, 103) that appeared to be citrate based on spectral data and sensitivity of the material to citrate lyase (20). All three lobes of the prostate and the seminal vesicles but not the coagulating gland of the rat contained citrate (73), but prostatic secretion from the dog did not (37).

FUNCTION Although the association of Zn with sperm and with the prostate has been studied for many years (45, 183), the function of Zn is still not clear (for review, see 54). Zn concentration decreased during epididymal transit in rats (47, 170), and its removal has been implicated in maturation of sperm. Since epididymal sperm is fertile (6), and prostatectomy did not affect fertility in the rat or guinea pig, the suggestion that the high concentration of Zn in the prostate is an evolutionary residue that no longer has a function (45) has some merit. Zn, in fact, inhibited fertilization by epididymal mouse sperm (10) although it did not affect the ability of human sperm to fertilize zona-free hamster ova (34). On the other hand, Zn had marked effects on the stability of sperm. Preincubation of sperm with Zn but not Ca or Mg markedly increased the stability of sperm as measured by resistance to swelling in sodium dodecyl sulfate (SDS) (33, 109–111). Zn also prevented head-tail detachment in SDS (32).

Because a postulated role for Zn was to maintain a quiescent state in sperm by inhibiting sperm metabolism, Zn concentration was determined in the reproductive tracts of bats, in which sperm is stored in an inactive state during hibernation. Zn concentration varied seasonally but was not higher during hibernation as would be expected if its function was to maintain quiescence. Zn concentration was two to three times higher than equivalent tissues in the rat or rabbit, however (54).

Zn Deficiency

While the need for high levels of Zn in prostatic fluid is not clear, there is no question of the importance of Zn for sexual development and function. Low Zn intake by young males of several species, including humans, interfered with normal sexual development (183). Exactly what the specific role of Zn is in addition to its effect on growth and food intake is not clear since many of the effects observed in Zn-deficient males were also observed in other types of undernutrition (77, 116).

TESTES In the young rat severe Zn deficiency had a marked effect on testicular weight, clearly greater than the effect of restricted feed intake (50, 114, 131, 160). Part of the difference was probably due to the more efficient feed utilization of +ZnRI rats, which, as discussed above, usually resulted in more growth in these animals. In mildly Zn-deficient rats in which body weight was only 30% less than that of +Zn rats, testicular weight was not affected. In pigs and guinea pigs testicular weight in −Zn and +ZnRI animals was not different (19, 89, 188). The marked reduction in testicular weight, which presumably reflected greater impairment of testicular function, was probably responsible for the greater reduction in testicular Zn concentration in the rat than in the pig. Surgical cryptorchidism reduced testicular Zn in rats to 50% of the controls (49), which suggests that Zn was reduced in nonfunctional testes irrespective of Zn intake. Zn concentration was reduced 25–50% in −Zn rat testes (23, 53, 114, 121, 131, 162) but less than 15% in the pig (89, 188).

Testicular lesions in young −Zn rats were similar to those of experimental cryptorchidism: severe atrophy of seminiferous tubules, sperm with malformed tails, and lesions of the axoneme (59). In mildly Zn-deficient rats in which testicular weight was not reduced, epididymal sperm numbered only 35% of that from +Zn rats, motility was reduced, and the sperm had various defects. Zn concentration of the sperm was not affected (187). Seminiferous tubules and germinal epithelium were not affected in young −Zn pigs, but Leydig cells had disordered smooth endoplasmic reticulum and accumulations of fat droplets (89). Lipid also accumulated in Leydig cells of the mouse before changes were apparent in the seminiferous tubules (176). Since Leydig cells are a major source of testosterone in testes, the greatly reduced activity of desaturases in testes of −Zn rats (23) was probably related to changes in these cells. Desaturase activities were decreased by castration, and testosterone injections restored the activity in both −Zn and +ZnRI castrates (53). In spite of large differences in enzymatic activity, only small changes in fatty acid composition of microsomal membranes were observed (23).

The effect of low Zn intake varied more in older than in young males. In both the rat and boar, large individual variation in testicular damage occurred that was not correlated with growth (121), duration of feeding, or Zn concentration of serum or organs (188). After four months of low Zn intake, testicular weight was reduced 30% in year-old rats (161). In contrast to the young −Zn rat in which the testes appeared normal after Zn repletion, testicular degeneration of mature testes did not appear to be reversible with Zn repletion (121). Testicular morphology was normal in adult rams given a low Zn diet, but lesions of the axoneme were present in the sperm (59). Sixty-day-old mice were sterile after three weeks of low Zn intake (176).

Five human males, average age 57 years, given low Zn intakes of 2.7–5 mg daily for 24–40 weeks had reduced sperm counts and reduced serum testoster-

one during the Zn repletion period but not during the period of Zn restriction; sperm motility and morphology were not affected (1). Changes in sperm count and reproductive hormones may have been related to body weight loss in these subjects, which was not regained during the repletion period (148).

PROSTATE Weights of the prostate and other accessory sex organs were reduced in Zn deficiency (50, 160, 161, 187) as they were in other nutritional deficiencies (116). A large part of the effect may have been due to effects on the testes, particularly on production of testosterone by Leydig cells. Testosterone affected not only weight but also Zn concentration of the prostate in Zn-adequate as well as Zn-deficient animals. Castrated rats (178) and mice (177) had reduced prostatic Zn concentrations as well as reduced weights, both of which were restored by injections of testosterone or dihydrotestosterone. Testosterone injections in intact immature rhesus monkeys also increased prostatic Zn (171). The lower concentrations of Zn in prostatic tissue from young boys and from men with chronic debilitating diseases were attributed to low serum levels of testosterone (115, 179). Factors other than testosterone must be responsible for prostatic Zn concentration in older men with decreased androgen levels, however, since concentrations did not decrease as would be expected (180).

Although serum testosterone was decreased in −Zn rats, the histological appearance of the prostate was generally similar to that of +ZnRI rats. In spite of the much lower Zn concentration of the glands from −Zn and +ZnRI rats compared to that of normal rats, prostatic architecture and epithelial differentiation developed normally even though the glands were much smaller than those of normal rats. Injection of testosterone in −Zn rats increased the size of the prostate and restored the histological appearance to that of normal rats. The high concentration of Zn in the prostate seemed therefore to be related to prostatic secretion rather than to a high cellular requirement (50).

Since much of the Zn in seminal plasma comes from prostatic fluid, the reduction in Zn concentration of seminal plasma by more than 50% after even a short term of low Zn intake in adult boars probably reflected an early effect on prostatic secretion; prostatic histology was not altered (188).

REPRODUCTIVE HORMONES In light of the disruption of Leydig cell morphology in −Zn animals (89, 176), one might expect a decrease in testosterone concentrations in testes and serum. Neither testosterone nor dihydrotestosterone concentration were decreased in testes of −Zn compared to +Zn rats, however (131, 162). Serum testosterone was generally (50, 131, 160, 162) but not always (114, 160) reduced. Serum testosterone was usually reduced in +ZnRI rats as well. Serum dihydrotestosterone was reduced in both −Zn and +ZnRI rats (131) in one study but was not affected by low Zn intake in another

(162). The decreased weights of accessory sex organs such as the prostate and seminal vesicles in −Zn and +ZnRI rats were also evidence of reduced androgen secretion by the testes since these organs are dependent on testicular hormone secretion. Human males had lower basal levels of testosterone and lower testosterone response to gonadotropin-releasing hormone (GnRH) injections during Zn repletion than during Zn depletion (1). Serum testosterone response was also slightly less in young −Zn rats after GnRH injection than in +ZnRI (114), but the fact that the testes did respond to the stimulus by increasing testosterone secretion indicated that the testes were able to respond to a sufficiently strong stimulus. Because binding of testosterone and dihydrotestosterone by crude rat testicular homogenates was not different, presumably the androgen-binding protein, which conveys androgen to Sertoli cells (site of sperm production), was not affected by Zn deficiency (131).

In addition to its effect on accessory sex organs, decreased serum testosterone concentration would reduce feedback inhibition of the pituitary and might then increase serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) as does castration (6). Serum LH was not different (160), however, or was decreased (114) in −Zn rats or rats with low protein intake with or without adequate Zn (162). Serum LH was unchanged by Zn status in adult humans (1). Because of the pulsatile nature of LH secretion (6), single measurements of LH concentrations may have been inadequate for detecting a difference; but serum LH was also unaffected or reduced by other types of undernutrition (77). Serum FSH was increased in young −Zn rats (114, 160, 162) but not in adults (160). Serum FSH in adult humans was also unaffected by Zn restriction or repletion (1). Pituitary concentrations of LH and FSH were increased in both −Zn and +ZnRI male rats (160). Serum LH and FSH concentrations were increased to a greater extent after GnRH injection in −Zn than in +ZnRI rats (114); hypothalamic GnRH was not different (160).

Similarly variable effects on hormone concentrations have been reported after various types of undernutrition in the rat (77). The variations probably reflected variations in the degree of malnourishment or, in the case of young males, the stage at which sexual maturation was interrupted. It is clearly advantageous for the animal to be able to reduce expenditure of energy on reproductive function if nutrient supply is limiting, but in spite of intensive study the mechanism by which reproductive function is reduced in undernourished animals is still not known. Since in −Zn rats the administration of GnRH resulted in increased serum LH, FSH, and testosterone (114), the pituitary and testes were able to respond appropriately to a sufficiently strong stimulus. That suggests either an impairment in hypothalamic GnRH or decreased sensitivity of the target organs. Strong evidence for reduced target organ sensitivity in undernourished animals is lacking. Since hypothalamic concentrations of GnRH were not different in −Zn and +Zn rats (160), impaired release is a possibility. If release is impaired, the question remains of

what inhibits release of hypothalamic GnRH in animals with inadequate Zn or other nutrients.

Summary

Male reproductive organs, particularly the prostate, contain considerable Zn. The high concentration in the prostate was not a cellular requirement but was associated with prostatic secretion. Since epididymal sperm are fertile and prostatectomy did not interfere with reproduction, the role of Zn in prostatic secretion is obscure, although Zn in vitro affected sperm stability.

The association of Zn with specific proteins in rat sperm tail indicated a requirement for Zn in sperm structure. Sperm with tail deformities as well as reduced numbers of sperm were produced by mildly Zn-deficient rats in which testicular weight was not affected. Zn deficiency in young males given very low Zn intakes resulted in testicular lesions and reduced accessory gland weights, largely as a result of reduced feed intake and/or reduced growth. The few studies of older animals indicated that effects of low Zn intake were more variable after sexual maturation. Testicular damage in older rats did not appear to be as effectively reversed by Zn repletion as it was in younger animals.

CONCLUSION

After much study it is still not clear to what extent Zn deficiency may be involved in reproductive problems in humans. Until better methods of assessing Zn status are available, the question is unlikely to be resolved. While decreased serum Zn concentration is normal in pregnancy, more information is needed on the extent of the decrease at various stages of pregnancy. The demonstrations in several species of severe effects of Zn deficiency on the fetus emphasize the importance of adequate Zn status in pregnant women. Women appear to be able to maintain adequate Zn status with intakes significantly below the 20 mg daily currently recommended for pregnant women, however. The suggestion that defective sperm may be produced under conditions of mild Zn deficiency deserves further study, as does the effect of Zn deficiency on adult males.

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