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Sara Maria Vieira Bernardo

Défice de Zinco no Desenvolvimento Fetal: Uma Revisão Sistemática

Zinc Deficiency in Fetal Development: A Systematic Review

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TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Zinc Deficiency in Fetal Development: A systematic review

ORIENTADOR

Carla Maria de Almeida Ramalho

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ZINC DEFICIENCY IN FETAL DEVELOPMENT: A SYSTEMATIC REVIEW

DÉFICE DE ZINCO NO DESENVOLVIMENTO FETAL: UMA REVISÃO SISTEMÁTICA

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ABSTRACT

Overview and Aims: Zinc is an essential micronutrient for many processes in human body. Zinc Deficiency (ZnD) is a prevalent condition and maternal ZnD before and during pregnancy interferes with fetal growth and development. Our aim was to evaluate the effect of maternal ZnD during pregnancy on the development of the fetus.

Study Design, Population and Methods: A systematic review of the published data on the association of maternal ZnD and fetal malformations was carried out by searching on PubMed, Web of Science and Scopus databases. A total of 10 studies were included in this review, eight been animal studies and two human studies.

Results: All the studies reported the association between maternal ZnD and deformed embryos and embryonic growth retardation, but only one provided the cut-off value of normal serum zinc levels. Under ZnD conditions, four studies found smaller fetuses, poor yolk sac circulations, placental abnormalities, and problems in the rotation of the embryos. Five studies reported skeletal, heart and neural tube defects. One study showed the relation between feeding cycles of animals under ZnD diets and the incidence of congenital anomalies. Also, one study reported an increased surface microvilli in ZnD embryos cells, as well as the presence of blebbing.

Conclusions: Prevalence of ZnD varies greatly in the literature due to the absence of standardized ZnD value and to differences existing between countries around the world. Maternal ZnD severely influences the embryofetal development. Nonetheless, further investigation regarding the impact of ZnD in humans would be beneficial to confirm and better comprehend these results.

Keywords: zinc, zinc deficiency, pregnancy, fetal anomalies, congenital malformation

RESUMO

Visão geral e objetivos: O zinco é um micronutriente essencial para muitos processos no organismo. A deficiência de zinco (DZn) é uma condição prevalente e a DZn materna, antes e durante a gravidez, interfere com o crescimento e desenvolvimento fetal, estando intimamente relacionada com malformações congénitas. O nosso objetivo é avaliar o efeito da DZn materna no desenvolvimento fetal durante a gravidez.

Desenho de estudo, População e Métodos: Realizámos uma revisão sistemática dos dados que associam a DZn materna e malformações fetais com pesquisas nas bases de dados PubMed, Web of Science e Scopus. Um total de 10 estudos foi incluído nesta análise, sendo oito estudos animais e dois estudos realizados em humanos.

Resultados: Todos os estudos reportaram uma associação entre a DnZ materna, embriões deformados e atraso no crescimento embrionário, mas apenas um forneceu o valor dos níveis séricos normais de zinco. Em condições deficitárias, quatro estudos encontraram fetos de reduzidas dimensões, sacos vitelinos com circulações pobres, anomalias placentárias e problemas na rotação dos embriões. Cinco estudos reportaram malformações esqueléticas, cardíacas e defeitos do tubo neural. Um estudo demonstrou relação entre ciclos de alimentação dos animais sob dietas pobres em zinco e a incidência de anomalias congénitas. Além disso, um outro estudo revelou um aumento de microvilosidades na superfície de células de embriões deficientes em zinco, bem como a presença de protuberâncias nas membranas plasmáticas.

Conclusões: A prevalência da DZn varia muito na literatura devido à ausência de um valor padronizado e às diferenças existentes entre países. A DZn materna influencia gravemente o desenvolvimento embrionário. No entanto, uma investigação mais aprofundada sobre o impacto da DZn em humanos seria benéfica para confirmar e compreender melhor estes resultados.

Palavras-Chave: zinco, deficiência de zinco, gravidez, anomalias fetais, malformações congénitas

INTRODUCTION

Zinc deficiency (ZnD) is one of the most prevalent micronutrient deficiencies in low-income countries, being present in high-income countries too.[1-3] The prevalence of inadequate zinc intake is estimated to be about 40% worldwide, ranging from 4 to 73% by sociodemographic factors.[4-6]

Zinc is considered an essential nutrient for many processes in human body. It is a substrate and cofactor for the activity of approximately 100 enzymes, being required for multiple functions.[2,3,7-9] This element plays a pivotal role in cellular replication, as many enzymes involved in DNA, RNA and protein synthesis depend on zinc to function. Multiple organs and systems can be affected by this deficit, including skeleton, brain, and lungs. Particularly, heart development can be sensitive to deficient levels of zinc.[4,10] We also know that zinc supports the immune system, has a central role in embryogenesis, and some antioxidant functions, being essential for normal fetal growth.[1,2]

During pregnancy, nutrient metabolism and energy requirements are greatly affected by physiological changes, and maternal zinc levels could drop by up to 35% in this period.[11] The mother nutritional status before and during pregnancy is a critical factor for fetal growth and health during and after pregnancy. It is known that malnourished mothers are more likely to have children with growth restriction and other prenatal deficiencies.[1,4]

ZnD in pregnant animals limits fetal growth and, if severe, has been shown to be teratogenic in rats, as well in monkeys.[1,3,7,12-16] Also, it has been implicated in the induction of fetal abnormalities in humans.[12] Numerous studies have shown that a ZnD diet fed to rats during pregnancy results in many types of malformations in a high percentage of developing fetuses. Also, even transitory ZnD is capable of causing severe malformations.[13,14,17,18] It is thought that it is because female rats fed with a ZnD diet during pregnancy are not able to mobilize enough zinc from body tissues, to meet the fetus normal development needs.[14]

When macroscopically evaluated, embryos exposed to a zinc-poor environment develop severe neural tube defects as well as abnormalities in a large majority of organs.

The aim of our study is to describe and evaluate the effect of maternal zinc deficiency during pregnancy on the fetus development.

METHODS

We performed a systematic review in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidance. We began with a literature review searching in three databases: PubMed and Scopus using the query "zinc deficiency AND fetal development AND fetal anomaly AND pregnancy" and Web of Science using the query "zinc deficiency AND pregnancy AND fetal outcome".

The search occurred between March and August 2021. Studies were included if they were an animal or human study, a randomized or observational prospective design, a clinical trial, and if they reported fetal anomalies caused by zinc maternal deficiency during pregnancy. We excluded studies if they were a systematic review or a letter, if fetal development anomalies were not correlated with ZnD and if were not written in English or Portuguese. No articles were excluded based on publication date. We assessed trials that evaluate ZnD in relation to pre conceptional phase, fetal development, as well as the impact of maternal factors in the supply of zinc.

This query resulted in a total of 74 articles on the PubMed database, 13 at Scopus and 89 on the Web of Science database. All the details of the search strategy are described in the Figure I. The selection and analysis of the articles were done by one independent reviewer that extracted pre-defined study characteristics into a structured table (author, publication year, country, study design, sample size, aim of the study, results, and conclusions) from each included article. Doubts were discussed with the second reviewer when necessary.

The risk of bias of each study included in this review was assessed using the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool for Human and Animal Studies that includes a questionnaire aimed to study risk of bias in several domains. Potential source of bias was graded as definitely low (++), probably low (+), probably high (-) or not

reported (NR), and definitely high risk of bias (--). Different points of view regarding the quality of articles were solved by consensus between the authors.

RESULTS

A total of 176 studies were identified through database searching. After exclusion of duplicates, we initially obtained 165 articles and select 35 of them, from the title and abstract analysis. From those, we excluded 19 for study type and language reasons. A full text analysis was subsequently performed, and 10 studies were eligible for inclusion (Fig. 1). The characteristics of all studies included in this review are available in Table I.

Of the 10 studies considered eligible, there were 8 interventional animal studies, 1 case control study and 1 cross-sectional study, both in humans. Articles were published between 1966 and 2021 and conducted in USA, Indonesia, Australia, and Republic of Iran.

Zinc Deficiency

Of the 10 studies included, only one[11] provided the definition of normal serum zinc levels based on a cut-off value of ≥ 56 μg/dL. In this study[11] and in Golalipour et al[3], maternal serum zinc levels were measured from blood samples (Table II). In all animal studies included, the authors manipulated the diet to make intentionally zinc deficient. Zinc's deficit official value was not the same in all the studies evaluated (Table III).

Anomalies due to Zinc Deficiency

1. Growth and morphological development

Hurley et al.[17] showed that animals receiving a zinc free diet did not grow and under extreme ZnD (0 ppm) fertility was not possible. After inducing a less severe deficit state (9 ppm), reproduction was able to occur, but they obtained a great number of abnormal fetuses. Rats receiving a ZnD diet also had a lower ratio of fetuses per pregnancy and more stillborns. Also, the majority (98%) of the born fetuses had gross congenital malformations (Table I).

Despite zinc being considered an essential nutrient for fertility, the effects of pre-conceptional ZnD of post-implantation development are sparce. Tian et al.[8] contributed by evaluating the impact of preconception ZnD on post implantation development, founding a high incidence of pregnancy losses and lower length values throughout pregnancy. To determine if implantation failure and development defects were consequence of the uterus inability to support implantation or by defects in the embryo itself, blastocyst embryos were transferred to pseudopregnant recipient females. On the 12.5th day of gestation (D12.5), placenta weight decreased 37% in ZnD animals, embryos decreased of 13% in the crown-to-rump length and 34% of implantation sites had no/viable embryo, compared to 7% in the C group (Table I).

Record et al.[7] studied the outcome of normal and morphologically imperfect embryos cultured under varying conditions (serum obtained from ZnD rats or zinc replete [ZnR] serum). On D9.5 gestation, there was normal embryo growth and development, in both ZnD and ZnR groups. The ZnD embryos were divided into two groups, according to their microscopic evaluation: normal appearance versus underdeveloped (in size and gestational differentiation). Apparently unaffected animals grew normally in both ZnD and ZnR medium. Those with previous identified anomalies, had retarded growth and developed multiple major abnormalities (poor yolk-sac circulation, failure in the formation of the chorio-allantoic placenta, and in the rotation to the dorsally converse position) (Table I).

2. Skeletal system defects

Lopez et al[4], realized that half of the litters from ZnD dams had at least 1 malformed fetus, and the longer the duration of the deficiency, bigger the number of abnormal fetuses. Their severity was correlated with gestation ZnD duration, being described limbs (deformed or duplicated, missing/fused digits) and tail (short/curly) defects, on 15th and 18th gestation days. Tian et al.[8] observed that ZnD animals had splayed digits on D16.5 of gestation versus the parallel arrangement of digits in the C group (Table I).

Hickory et al[14], investigated the effect of moderate maternal ZnD (1.3 ppm of Zn) on the fetal skeletal system development. They found that skeletal malformations were primarily

limited to vertebrae, ribs, and long bones. These fetuses had several anomalies of trunk and limbs, specially of the hind limbs, that appeared small and atrophied. Also, 15% of the fetuses had kyphosis with or without scoliosis. Overall examination of the fetuses showed significantly less bone ossification, more evident in cranial bones.

In the study of Hurley et al.[17] skeletal malformations were also common: scoliosis or kyphosis, short or missing mandible, clubbed forefeet and hindfeet, fused or missing digits, and curly or stubby tail were present (Table I).

3. Heart defects

The teratogenicity observed in fetuses from ZnD dams involved vascular segments and heart structures. The normal development of these structures depends on the appropriate infiltration and differentiation of cardiac neural crest cells (NCC), so Lopez at al.[4] examined whether changes in these cells and increased cell death could play a role in ZnD-induced heart defects. In the scanning electron microscopy (SEM) was observed abnormal heart development in many of the ZnD fetuses. Large proportion of the fetuses with severe anomalies in the aortic sac, double outlet right ventricle, anomalies of the outflow tract and the great vessels; irregularities in ventricular trabeculation, anomalies of the ventricular septum and atrioventricular valves formation were noted. Also, a few subclavian and carotid arteries were small or missing. Alignment anomalies and persistent truncus arteriosus were present too.

4. Neural Tube defects

Despite the supplementation with folic acid in pregnant women, neural tube defects (NTD) continues to be one of the most frequent congenital malformations in the world. Golalipour et al.[3] determined the role of maternal ZnD in NTD neonates. The most frequent type of NTD was spina bifida followed by anencephaly and encephalocele. In the study of Tian et al.[8] there was a developmental delay in the ZnD animals, with a larger fourth ventricle at D12.5 of gestation, consistent with NTD. Later, at D16.5, 57% of ZnD embryos had a pronounced bump on the back of the head.

5. Other defects

Lopez et al.[4] found anomalies in the brain on D13.5 of gestation of ZnD embryos and some anomalies in the face: coloboma, cleft palate and microphthalmia. In addition, Hurley et al.[17] found another type of malformations: hydrocephalus or hydranencephalus; cleft palate; herniations; heart abnormalities; lung abnormalities; small or missing eyes and urogenital abnormalities (Table I).

Rohmawati et al.[11] assessed the correlation of maternal serum zinc levels with cord blood P1NP (Procollagen type I Intact N-terminal Propeptide- specific bone growth marker to measure fetal ossification) levels and anthropometric measurements of newborns. In their study, more than half of pregnant mothers had low serum Zn levels (< 56 µg/dL). They found a significant correlation between maternal serum zinc levels, newborn weight and length at birth, as well as head circumference. A positive and significative correlation between maternal serum zinc levels and cord blood P1NP levels was also present (Table I).

Zinc Deficiency between 8th and 11th gestation days: a critical period

1. Fetal malformations

Between the 8th and 11th rat's gestation days is when closure of neural tube occurs, being a period of greatest significance to congenital malformations. Harding et al.[12] examined the ultrastructural effects of ZnD during this critical period of embryogenesis. They found a strong association between low maternal serum zinc levels (Table III), reduced embryonic sizes, total body length, cardiac and head diameter, head height, and increased teratogenesis (Table I).

In the study of Record et al[13], 54% of the ZnD embryos had at least one observable malformation, such as deficient yolk sac circulation, failure of allantoic fusion, incomplete flexion, anophthalmia, heart defects, and absence of forelimbs. Embryonic size, number of somites and protein content were significantly reduced in ZnD dams. Growth retardation was evident in all the ZnD embryos, and almost all the malformed embryos had some form of defect

involving the head region: reduced prosencephalic, telencephalic and mesencephalic development; in 12% of all malformations was failure of the neural tube failed to close completely.

1.1. Feeding cycles

In a study involving similar animals confined to metabolism cages, Record et al.[13] noted that after 2-3 days with the ZnD diet, each animal entered in a cycle of feeding or fasting. They compared these feeding cycles with the time of organ systems development and suggested that a period of "feeding" (and low maternal zinc levels) at 8th and 9th day of gestation could be associated with major malformations identified at D11.5. Later, they concluded that high intakes of the ZnD diet at D8-9 of gestation increased the uniformity between litters. The embryos were significantly retarded in all growth parameters measured, the incidence of open neural tube increased, reporting that 72% of the embryos had at least one major malformation.

2. Histology anomalies

Harding et al.[12] identified differences in the ZnD embryos cranial epithelium, such as denser microvilli around the border of the squamous epithelial cells and increased blebbing. These alterations reflect a cellular effort to potentiate Zn absorption, in a ZnD environment. There were also described various dead or dying cells in the neural tube epithelium.

Risk of Bias

It was only possible to evaluate confounding bias in 2 studies, as most articles included were animal experiments. Six studies may contain selection bias because the allocation to study groups was not concealed. The same 6 studies were considered to have performance bias, because they were not doubled blinded. Eight studies were considered to have probable risk of detection bias, and three studies had probable risk of potential threat to internal validity. The evaluation of the risk of bias assessment of these studies is present in Table IV.

DISCUSSION

Maternal ZnD is a common condition in the world, with a wide prevalence described in literature, due to: absence ZnD value consensus and discrepancy between low- and high-income countries epidemiological data. Only one study[11] in this systematic review included the value that defined ZnD.

Many studies reported an increased risk of congenital anomalies, fetal growth retardation and placental dysfunction, if there is a ZnD during pregnancy. However, the reason why the zinc deficit causes these anomalies is not known yet.

We observed that ZnD is related to a poor yolk sac circulation[7,13], failure at formation of a chorio-allantoic placenta[7], failure of the embryo to rotate to a converse position[7], a high prevalence of smaller fetuses per litter[8,11,19] and a major number of fetuses with gross congenital malformations[3,4,8,14,17,19]. The most prevalent malformations found were skeletal, heart and neural tube defects.

Two studies[12,13] reported pregnancy and fetal alterations due to ZnD between the critical period of embryogenesis, consistent with the malformations reported by other authors. Curiously, they found that animals under a ZnD regime adopted a cyclical feeding pattern with phases of feeding or fasting. In this way, they regulate their serum zinc levels through their food intake: high intake of food between 6^{th} and 7^{th} gestation days leaded to unsuccessful implantation and embryos losses; feeding between 8^{th} and 9^{th} was associated with multiple gross abnormalities. The authors of this study postulated that ZnD animals alternate between a catabolic state (fasting) when maternal tissues release zinc to the serum and an anabolic state (feeding) when there is a zinc uptake, from the serum to the maternal tissues. Therefore, they concluded that even transitory and brief ZnD episode can deeply affect fetal development. Finally, Harding et al[12] reported that cranial epithelium cells from ZnD embryos had an increased population of microvilli around the border of the squamous cells. This could be a way for these cells to extract from the amniotic fluid as much zinc as possible. Increased blebbing was also evident in the surface of these embryos which can reflect cells under stress and could be a method for the removal of waste that cannot be adequately metabolized.

Also, the results of one study[7] indicated that ZnD teratogenic effects cannot be induced by culture of ZnR embryos in ZnD serum, suggesting that maternal ZnD is able to irreversible induce teratogenicity, even before the 9.5th gestation day.

With this review we can conclude that maternal ZnD can strongly influence the embryofetal development, and the longer the duration, greater the number of abnormalities. Also, an extreme ZnD can even prevent reproduction from occurring.[17]

The inclusion of animal and human studies could be considered a strength of our study. However, the inclusion of only two human studies is a limitation. Another strength of our research is the absence of limitations in publication date and the use of three databases, trying to cover the maximum knowledge existent. Additionally, the quality assessment was done using OHAT Risk of Bias Tool. Generally, the studies included have a low risk of bias, concluding that they have quality. Also, the characteristics of the individual studies were described in a table, in an effort to present the data in a more transparent and comparable way.

Other important limitations of this review were the small sample size and the differences between the authors about the zinc levels measurements and cut-off values. It is essential to standardize the criteria considered in the definition of ZnD to enable comparison between studies.

To better comprehend the impact of this micronutrient deficiency on pregnancy, we suggest that more studies should be conducted, specially in humans.

DISCLOSURES

The authors declared no financial support or competing interests.

AUTHORS CONTRIBUITION

Sara Bernardo: Conceptualization, Data curation, Investigation, Methodology, Writing- original draft, Writing-review and editing

Carla Ramalho: Data curation, Methodology, Validation, Writing- review and editing

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Table I. Characteristics of included studies.

Author, year, country	Type of study	Objective	Results	Conclusions
			Animal Studies	
Hickory et. al[14], 1979, USA	Interventional Study	Investigate the effect of moderate ZnD on the development of the fetal skeletal system.	Malformations found in ribs (59%), rudimentary ribs (28%), hemivertebrae (50%), and underdeveloped tibia and fibula (23%). Scoliosis was clear in several fetuses.	Skeletal malformations found were limited to vertebrae, ribs, and long bones. Overall calcification and ossification of cranial and long bones appeared delayed or disturbed.
Lopez et al[4], 2008, USA	Interventional study	Examine whether increased cell death and changes in cardiac NCC play a role in ZnD-induced heart defects.	No. of resorptions D15.5: 1.87±0.60 (ZnD) vs 0.18±0.12 (C). No. of malformed fetuses D18.5: 3.07±1.08 (ZnD) vs 0 (C). Anomalies of the brain (7.7%) and facial (1.0%) defects D13.5; limb (14.3%) and tail (12.6%) anomalies at day 15; limb (4.4%) and tail (20.7%) anomalies at D18.5.	The longer the duration of the ZnD the greater the no. of abnormal fetuses. The types and severity of the anomalies varied with the duration of the gestational ZnD. This study supports that severe maternal ZnD can result in cardiac anomalies: teratogenic outcomes involved heart structures and vascular segments. The development of structures that depend on the proper migration of NCC seems vulnerable to these changes.
Harding et al[12], 1987, USA	Interventional Study	Extend earlier findings about cell death in the NT of embryos exposed to severe ZnD and examine the ultrastructural effects of ZnD during 8-11 days of gestation	Crown-rump length: 1.58±0.09 mm vs 1.78±0.08 mm (ZnD vs ZnR) Total body length: 4.04±0.34 mm vs 5.52±0.17 mm Cardiac diameter: 0.82±0.04 mm vs 0.98 ± 0.04 mm Head diameter: 0.75±0.04 mm vs 1.02±0.04 mm Head height: 0.72±0.04 mm vs 0.95±0.04 mm Teratogenesis: 30% of the ZnD failed to rotate and 40% had tail defects (0% ZnR). 53% of the ZnD and 20% of the ZnR had an open NT.	Reduced growth and development were evident. Increased fetal death was not associated with ZnD at this point of pregnancy. Ring of microvilli and increased blebbing reflects cells adaptation to an abnormal environment. Abundance of dead or dying cells within the neural epithelium of the ZnD embryo: the result of the effects of ZnD upon cellular membranes.
Record et al[7], 1985, Australia	Interventional study	Determine the outcome of morphologically imperfect embryos removed from ZnD damns and cultured under varying conditions in vitro	ZnD group: -no. embryos w/satisfactory yolk sac circulation: 10 ZnD vs 39 ZnR; -no. embryos which rotated dorsally was 11 ZnD vs 37 ZnR -no. embryos w/chorio-allantoic placenta was 12 ZnD vs 38 ZnR RD group: -no. of embryos: 20 (RR) vs 28 (RD) -embryonic protein: 123.2±8.3 (RD) vs 102.1±4.4 (RR) µg/embryo -yolk sac protein: 103.0±3.5 (ZnR) vs 89.5±5.6 (ZnD) µg protein/embryo DaD group: -embryos with satisfactory yolk sac circulation 43% -embryos which rotated 24% -embryos w/chorioallantois placenta 52% vs 96% RR group -open NT 57% vs 3.6%(RR) DnD group: -embryos with satisfactory yolk sac circulation 100% -embryos which rotated 90%	It is apparent that in vitro the embryo can extract sufficient zinc even from ZnD medium for normal cell growth. The results indicate that maternal ZnD can exert an influence prior to day 9.5 of gestation. Poor or non-existent yolk-sac circulation, imperfect rotation of the embryo, failure of the chorio-allantoic placenta and partial or incomplete closure of the NT were the most common and serious abnormalities observed. The results from this study suggest that the teratogenic effects of ZnD cannot be induced by direct culture of zinc-replete embryos in zinc-deficient serum. Maternal ZnD can exert it's teratological influence prior to day 9.5 of gestation and that these effects are not easily reversible.
Record et al[13], 1986, Australia	Interventional study	Determine if some diet-related maternal metabolic factors might influence the	-Animals confined to plastic cages: 1.7% of all ZR embryos showed any defect. Yolk sacs diameter in ZnD: 2.6 ± 0.2 mm vs 3.6±0.1/4.0± 0.1 mm (ZR)	ZnD dams can regulate their food intake inducing large changes in the Zn levels. Production of specific malformation is linked intimately to the timing of the feeding and fasting stages of the cycle.

Author, year, country	Type of study	Objective	Results	Conclusions
		supply of Zn and hence embryonic development during the critical period of organogenesis.	Yolk sac protein in ZnD: 168 ± 5 µg vs 173 ± 5/159 ± 9 µg (ZR) No. of somites in ZnD: 20.9 ± 0.4 vs 24.3 ± 0.3 (ZR); protein content in ZnD 143 ± 6 vs 257 ± 10/239 ± 14 (ZR) Deficient yolk sac circulation: 28%; Failure of allantoid fusion: 33%; Incomplete flexion: 37%; Anophthalmia 26%; Heart defects: 5%; Absence of forelimbs: 18%; all associated with the smaller embryos. 26% with eye abnormalities; 18% with ear abnormalities -Animals fed to one of two schedules: 32% open NT 4/9 embryonic losses in dams fed to the inverse schedule.	High food intake on D6 and D7 show a significant loss of embryos without evidence of implantation. If feeding phase were at D8 and D9 (time of more susceptibility to teratogenesis) multiple gross malformations are produced. Embryos removed at D11.5 of gestation showed a high frequency of malformations of all organ systems. Growth retardation was evident in all the ZnD embryos, but the distribution of malformations was not uniform. Probably, during the feeding cycle, ZnD animals alternate between a fasting, catabolic state when maternal tissues release Zn to the serum and a feeding, anabolic state accompanied by a net uptake of zinc from the serum to the maternal tissues.
Hurley et al[17], 1966, USA	Interventional study	Study the effects of ZnD on embryonic development in rats	Net body weight change: -21g (ZD) vs +59 (ZSu) No. of implantation sites: 280 vs 226 No. resorptions: 152 (ZnD) vs 9 (C) No. abnormal fetuses with 9 ppm ZnD diet: 125 (ZnD) vs 0 (C) Gross congenital malformations: Cleft palate (34%); Scoliosis or kyphosis (47%); Short or missing mandible (28%); Clubbed forefeet (34%) or hindfeet (48%); Fused or missing digits (80%); Hydrocephalus or hydranencephalus (65%); Herniations (17%); Heart abnormalities (13%); Lung abnormalities (42%); Curly or stubby tail (53%); Small or missing eyes (35%) and Urogenital abnormalities (49%).	The immediate and complete response to supplementation with zinc established that the sole missing factor was this essential trace element. Almost all the full-term fetuses showed gross congenital malformations covering a wide variety of organ systems (skeletal, brain, eye, heart, lung, and urogenital defects). The fetuses from ZnD females contained less zinc than did their controls, suggesting that the congenital anomalies resulted from a direct effect of lack of zinc in the fetal tissue and not because of and indirect effect of the maternal metabolism on fetal development.
Tian et al[8], 2014, USA	Interventional study	Evaluate the impact of preconception ZnD on post implantation development.	Embryo weight: 23% D16.5 Length: 31% D10.5; 13% D12.5; 10% D16.5 Pregnancy losses: 46% D10.5; 34% D12.5; 51% D16.5 Placenta weight on D12.5: 30 mg in ZnD group vs 48 mg in C group Delayed or aberrant NT development on D16.5: 41-57% ZnD embryos when transferred to normal recipients: 38% smaller, implantation rate 10% vs 40% for C group Blastocyst outgrowth 56% in ZnD group vs 75% in C group Area occupied by differentiating trophoblast: 40% greater in C. Implantation sites: 29±2.5 (C) vs 17±2.9 (ZnD); $P<0.05$ Cross-sectional area: 36% smaller in the ZnD group (3.17±0,25 mm^2 (C) vs 2.03±0.21 mm^2 (ZnD); $P<0.05$)	Acute preconception ZnD severely affects post implantation development of the fetus and placenta, leading to a very high rate of pregnancy losses. This state results in multiple programing defects in the trophoblast cell lineage. ZnD doesn't need to be prolonged to have an impact on subsequent development, even after normal dietary zinc is restored. Fetal placenta is an important tissue affected by preconception ZnD. Zn is acutely important for ovarian function and the production of fertile oocytes and embryos because development delay in ZnD animals could be the result of poor oocyte/embryo quality and compromised uterine function.
Hurley et al[19], 1972 USA	Interventional study	Investigate the consequences of transitory maternal ZnD during gestation on postnatal growth, survival and Zn status of rats.	Birth weight of the offspring was significantly reduced and 13-35% of the pups were stillborn. More than 80% of the examined offspring from females given the transitory ZnD regimen were malformed. Defects observed: clef palate, clubbed feet, anophthalmia, hydrocephalus, anencephalus, syndactyly, hydroureter, and hydronephrosis. The survival rate was poor compared with C group (less than 29% on experiments A and C, and 46% on experiment B). They were weak at birth and mortality was greatest in the first 7 days of life.	The dietary Zn intake affects very quickly the level of zinc in the blood. The consequences of the brief deprivation of zinc are evident in the high proportion of stillborn young and the large no. of congenital malformations in the litters. Female ZnD rats gave birth to offspring reduced in weight and a high incidence of congenital malformations. Many of them were stillborn, and survival to weaning was poor. The stillbirths and gross malformations appear to be due to a specific effect of inadequate zinc supply to the embryo at this critical period, rather than to decreased food intake of the mother.

Human Studies

Author, year, country	Type of study	Objective	Results	Conclusions
Rohmawati et al[11], 2021, Indonesia	Cross-sectional study	Assess the correlation of maternal Zn with cord blood P1NP levels and anthropometric measurements of NB.	54.8% of pregnant mothers with low serum zinc levels. Significant correlation between maternal serum zinc levels and NB birth weight (r=0,648) and birth length (r=0,656), as well as head circumference (r=0,578; P=0,001). Correlation between maternal zinc levels, NB weight: (3323.8±389.3 kg, r=0,648), birth length (49.2±1.6 cm, r=0,656), and head circumference (33.9±1.2 cm, r=0,578; p=0,001). Correlation between maternal serum zinc levels and cord blood P1NP (3045.3±1287.1 µg/dL, r=0.469; P=0.002).	Low levels of maternal serum zinc are commonly found during pregnancy. There were positive significant correlations between maternal serum zinc levels and cord blood P1NP levels with anthropometric measurements of NB.
Golalipour et al[3], 2009, Islamic Republic of Iran	Case-Control study	Determine the role of maternal serum ZnD in NTD in neonates.	Spina bifida: 14 neonates (61%) vs anencephaly in 7 (30%) vs encephalocele in 2 (9%). Association between the presence of NTD and ZnD (OR 5.06; 95% CI: 1.51–16.94; *P* = 0.008).	The results of this study found an association between NTD in neonates and ZnD in mothers. NTD continues to be one of the most frequent congenital malformations around the world. Zinc supplementation should be considered for further decrease in recurrence and occurrence of NTD.

ZnD- Zinc Deficiency; NCC- neural crest cells; C-control; D15.5- 15.5th day; NT- neural tube; ZnR- zinc replete; RD- replete medium-deficient medium; RR- replete medium-replete medium; DaD- deficient abnormal-deficient medium; DnD- deficient normal-deficient medium; ZSu- zinc supplemented; P1NP- Procollagen type I Intact N-terminal Propeptide; NTD- neural tube defects; NB- newborns; NTD- neural tube defects.

Table II. Zinc levels considered in the human studies.

Author	Zinc level considered	Sample dosing	Cases	Controls	P
Rohmawati et al[11]	< 56 µg/dL	Maternal blood sample	54.8% ZnD	45.2% ZnD	_____
Golalipour et al[3]	< 10,6 mmol/L	Maternal blood sample	56.5% ZnD	19.4% ZnD	< 0.005

ZnD- zinc deficient**.**

Table III. Zinc levels considered in the animal studies.

Author	Weigh (g)	ZnD diet	ZnR diet	Sample dosing	Measurement day (gestation)	Amount of Zn in ZnD dams	Amount of Zn ZnR dams	P value
Hickory et al[14]	200-250	1,3 ppm Zn	100 ppm Zn	Maternal blood samples and amniotic fluid	15, 20 (blood)	D15: 58 µg/dL D20: 210 µg/dL	D15: 129 µg/dL D20: 134 µg/dL	D15: <0,025 D20: NS
					20 (amniotic fluid)	93 µg/dL	11 µg/dL	<0,05
Lopez et al[4]	160-180	< 1,0 µg Zn/g	25 µg Zn/g	Maternal blood samples	13.5	3.64±0.47 µM	18.90±0.42 µM	<0,05
					15.5	5.39±0.58 µM	19.83±0.62 µM	<0,05
					18.5	5.34±1.45 µM	15.65±1.07 µM	<0,05
Harding et al[12]	240	< 0,5 µg Zn/g	100 µg Zn/g	Maternal blood samples	11	0.88±0.07 µg/mL	1.43±0.18 µg/mL	<0,005
Record et al[7]	200-230	< 0,5 µg Zn/g	100 µg Zn/g	Maternal blood samples	9.5	0.56 µg /mL	1.29 µg /mL	_____
Record et al[13]	210-230	< 0,5 mg Zn/kg	100 mg Zn/kg	Maternal blood samples, fecal and urinary samples	11.5 (1st study, blood)	0.53±0.10 µg/mL	1.07±0.04	P<0.01
					11 (2nd study, urinary and fecal output)	U: 54±3 µg F: 1.177±199 µg	U: 66±4 µg F: 10.720±514 µg	_____
					8 and 9 (3rd study, blood)	1.13±0.04 µg/mL	1.04±0.05 µg/mL	_____
					6,7,10 and 11 (3rd study, blood)	0.5±0.06 µg/mL	1.06±0.03 µg/mL	P<0.01
Hurley et al[17]	?	9 ppm Zn	60 ppm Zn	?	21	40±3 µg/whole body	97±3 µg/whole body	P<0.001
Hurley et al[19]	210±10	0,4 ± 0,1 ppm Zn	100 ppm Zn	Maternal blood samples	8	53±5 µg Zn/100mL	138±12 µg Zn/100 mL	_____
					14	41±6 µg Zn/100 mL	124±8 µg Zn/100 mL	_____
Tian et al[8]	?	< 1 mg Zn/kg	29 mg Zn/Kg	_____	_____	_____	_____	_____

Zn-zinc; ZnD-zinc deficiency; ZnR-zinc replete; D-day; ?-no information about this.

Table IV. Risk of bias assessment using the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool for Human and Animal Studies Potential source of bias was graded as definitely low risk (++), probably low risk (+), probably high risk or not reported (- or NR) and definitely high risk (--).

Study	Study Design	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Did selection of study participants result in appropriate comparison groups?	Did the study design or analysis account for important confounding and modifying variables?	Were experimental conditions identical across study groups?	Were the research personnel and human subjects blinded to the study group during the study?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Were there no other potential threats to internal validity?
Hickory et al[14], 1979, USA	Animal Experiment	(+)	(-)	Not aplicable	Not aplicable	(++)	(-)	(NR)	(++)	(-)	(++)	(++)
Lopez et al[4], 2008, USA	Animal Experiment	(++)	(++)	Not aplicable	Not aplicable	(++)	(++)	(NR)	(++)	(-)	(++)	(++)
Harding et al[12], 1987, USA	Animal Experiment	(++)	(-)	Not aplicable	Not aplicable	(++)	(-)	(NR)	(++)	(-)	(++)	(++)
Record et al[7], 1985, Australia	Animal Experiment	(NR)	(NR)	Not aplicable	Not aplicable	(++)	(NR)	(NR)	(++)	(-)	(++)	(+)
Record et al[13], 1986, Australia	Animal Experiment	(++)	(-)	Not aplicable	Not aplicable	(++)	(-)	(++)	(++)	(-)	(++)	(-)
Hurley et al[17], 1966, USA	Animal Experiment	(NR)	(-)	Not aplicable	Not aplicable	(++)	(-)	(NR)	(++)	(-)	(++)	(-)
Tian et al[8], 2014, USA	Animal Experiment	(NR)	(-)	Not aplicable	Not aplicable	(++)	(-)	(+)	(++)	(-)	(++)	(-)
Hurley et al[19], 1973, USA	Animal Experiment	(NR)	(-)	Not aplicable	Not aplicable	(++)	(-)	(+)	(++)	(-)	(++)	(++)
Golalipour et al[3], 2009, Republic of Iran	Case-control study	Not aplicable	Not aplicable	(+)	(++)	Not aplicable	Not aplicable	(++)	(++)	(+)	(++)	(++)
Rohmawati et al[11], 2021, Indonesia	Cross-sectional Study	Not aplicable	Not aplicable	(++)	(++)	Not aplicable	Not aplicable	(++)	(-)	(+)	(++)	(++)

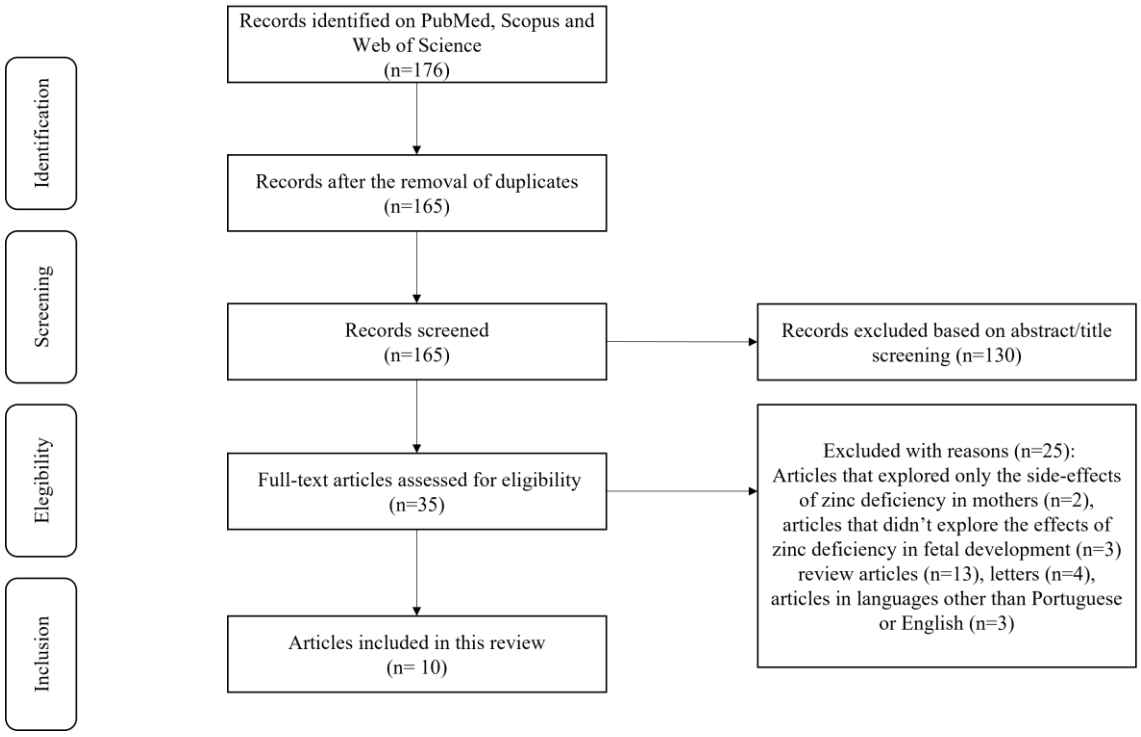

Figure 1. PRISMA flow diagram for included studies.

ATTACHMENTS

I- PRISMA 2009 checklist for systematic reviews.

Section/topic	#	Checklist item	Reported on page and paragraph/ table #
TITLE			
Title	1	Identify the report as a systematic review.	Page 6
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 7
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 9
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 10, 1st paragraph
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Not applicable
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 10, 3rd paragraph
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 10, 2nd paragraph
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Page 10, 2nd paragraph
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 10, 4th paragraph
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 10, 4th paragraph
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 10, 3rd and 4th paragraphs
Risk of bias in individual studies/ Risk of bias across studies	12/ 15	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 10, 5th paragraph
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Not applicable as this review does not include a meta-analysis
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not applicable as this review does not include a meta-analysis
Additional analysis	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, metaregression), if done, indicating which were pre-specified.	Not applicable as this review does not include a meta-analysis
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 11, 1st paragraph
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 11, 2nd paragraph

Risk of bias within and across studies	19/ 22	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 15, 3rd paragraph
Risk of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Not applicable as this review does not include a meta-analysis
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not applicable as this review does not include a meta-analysis
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, metaregression [see Item 16]).	Not applicable as this review does not include a meta-analysis
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 16, 3rd and 4th paragraph
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 17, 3rd and 4th paragraph
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 17, 5th paragraph
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data), role of funders for the systematic review.	Not applicable

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- **ARTIGO DE OPINIÃO**
- **CARTA AO EDITOR**

Uma subsecção dos artigos de opinião intitulada "**Para lá da Ciência**" permite a submissão de textos sobre a vivência pessoal na área da Obstetrícia e Ginecologia e sobre aspetos históricos da Obstetrícia/Ginecologia Portuguesa.

A revista publica também **Normas de Orientação Clínica** da responsabilidade das Sociedades pertencentes à Federação das Sociedades Portuguesas de Obstetrícia e Ginecologia (FSPOG).
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- **IMAGE OF THE TRIMESTRE**
- **OPINION ARTICLE**
- **LETTER TO THE EDITOR**

A sub-section of opinion articles entitled "Beyond Science" allows the submission of texts reporting personal experiences in the field of Obstetrics and Gynecology and historical aspects of the speciality in Portugal.
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que não pode exceder 300 palavras tratando-se de estudos originais e 100 palavras nos restantes. Este texto não pode incluir qualquer referência aos autores ou à instituição onde o estudo foi realizado. A estrutura é diferente de acordo com o tipo de artigo:

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- **ESTUDO ORIGINAL** – secções divididas com os títulos: **Introdução**, **Métodos**, **Resultados** e **Discussão**; dimensão máxima **3000** palavras.
- **ARTIGO DE REVISÃO** – estrutura livre; dimensão máxima **3000** palavras.
- **ARTIGO DE OPINIÃO** – estrutura livre; dimensão máxima **1500** palavras.
- **CASO CLÍNICO** – secções divididas com os títulos **Introdução**, **Caso Clínico** e **Discussão**; dimensão máxima **1500** palavras.
- **IMAGEM DO TRIMESTRE** – estrutura livre; dimensão máxima **500** palavras. Numero máximo de imagens: 2

4. As investigações que envolvem seres humanos ou animais devem incluir no texto a referência de que foram seguidas as normas éticas internacionais e da existência de aprovação prévia por uma **Comissão de Ética** apropriada (idealmente incluir o número da aprovação). No caso de casos clínicos ou imagens do trimestre é necessário que haja referência à obtenção de **consentimento informado** dos participantes.

5. As **abreviaturas** devem ser empregues com moderação e definidas por extenso aquando da primeira utilização, tanto no resumo como no texto principal do artigo.

6. Devem ser sempre utilizados os nomes genéricos dos **medicamentos**, excepto quando o nome comercial é particularmente relevante. Neste caso, devem ser acompanhados do símbolo ®.

7. Os **equipamentos** técnicos, **produtos** químicos ou farmacêuticos citados no texto devem ser seguidos entre parêntesis do nome do fabricante, cidade e país onde são comercializados.

8. No final do texto principal os autores podem incluir os **agradecimentos** que queiram ver expressos no artigo.

9. No final do texto principal os autores devem incluir a referência à existência ou não **de conflitos de interesse**.

10. No final do texto principal deve ser incluída a **contribuição individual de cada autor** para o artigo.

11. As **referências** deverão ser numeradas consecutivamente na be indicated.

5. Original studies, review articles, opinion articles, case reports and "images of the trimester" must include an **abstract in English**, which should not exceed 300 words for original studies and 100 words for all other submissions. The text must not include any reference to the authors or to the institution where research took place. The structure of the abstract varies according to the article type:

- **ORIGINAL STUDY** – paragraphs with the headings **Overview and Aims**, **Study Design**, **Population**, **Methods**, **Results**, and **Conclusions**.
- **OTHERS** – free structure.

6. Original studies, review articles, opinion articles and case reports must include 1-5 **keywords**, according to MeSH terminology (www.nlm.nih.gov/mesh/meshhome.html).

7. All articles must include a **title in Portuguese**, which cannot exceed 150 characters in length, including spaces.

8. All articles submitted as Case Reports and Images of the Trimester should be **entirely written in English.**

9. Authors should include a cover letter, which is an opportunity to introduce your study to the editor, highlighting the most important findings and novelty.

Preparation of the manuscript, tables and figures

1. Uploaded files containing the main manuscript, tables and figures must not contain any reference to the authors or to the institution(s) where research was conducted.

2. All texts should be submitted **double spaced**, using an **11-point Times New Roman** font.

3. The structure and maximum dimensions (excluding references) of the **main manuscript** vary according to the type of article:

- **ORIGINAL STUDY** – separate sections with headings: **Introdution**, **Methods**, **Results** and **Discussion**; limit of **3000** words.
- **REVIEW ARTICLE** – free structure; limit of **3000** words.
- **OPINION ARTICLE** – free structure; limit of **1500** words.
- **CASE REPORT** – separate sections with headings: **Introduction**, **Case Report** and **Discussion**; limit of **1500** words.
- **IMAGE OF THE TRIMESTRE** – free structure; limit of **500** words. Maximum number of images: 2.

4. All research involving human subjects or animals should contain the reference that international ethical standards have been followed and that have prior approval by an appropriate **Ethics Committee** (include approval reference). In clinical cases or images of the trimester, **informed consent** of the participants is required.

5. Abbreviations should be used sparingly and written in full extent at first usage, both in the article's abstract and in the full body of the text.

6. Drugs should always be referred to by their generic names, except when the trade name is of particlular relevance. In this case they should be accompanied by the symbol®.

7. Technical **equipment,** chemical or pharmaceutical **products** cited in the text should be followed in brackets by the name of the manufacturer, city and country where they are commercialised.

8. At the end of the main text, authors may include the **aknowlegments** that they would like published in the article.

ordem em que são mencionadas no texto, tabelas ou legendas de figuras, usando números arábicos em sobrescrito; exemplo [1,2,3]. Os artigos aceites para publicação, mas ainda não publicados, podem ser incluídos na lista de referências no formato habitual, usando o nome da revista seguido da expressão *in press*. As comunicações pessoais, resumos em livros de resumos de congressos, páginas web e artigos ainda não aceites não podem ser incluídos na lista de referências.

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- **ARTIGO DE REVISÃO** – máximo de 125 referências.
- **ARTIGO DE OPINIÃO** – máximo de 20 referências.
- **CASO CLÍNICO** – máximo de 20 referências.
- **IMAGEM DO TRIMESTRE** – máximo de 5 referencias

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- *Exemplo de* **artigos publicados em revistas**:
  Grant JM. The whole duty of obstetricians. BJOG 1997;104:387-92.
- *Exemplo de* **Capítulos de livros:**:
  Goldenberg RL, Nelson KG. Cerebral Palsy. In: Maternal-Fetal Medicine (4th Edition). Creasy RK, Resnik R (eds). WB Saunders;1999:1194-214.

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- **REVIEW ARTICLE** – maximum of 125 references.
- **OPINION ARTICLE** – maximum of 20 references.
- **CASE REPORT** – maximum of 20 references.
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- *Example of* **articles published in scientific journals**: Grant JM. The whole duty of obstetricians. BJOG 1997;104:387-92.
- *Example of* **Book chapters:** Goldenberg RL, Nelson KG. Cerebral Palsy. In: Maternal-Fetal Medicine (4th Edition). Creasy RK, Resnik R (eds). WB Saunders;1999:1194-214.

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