Does Suppression Levels of Testosterone Have an Impact in The Craniofacial Growth? A Systematic Review in Animal Studies

A supressão de testosterona impacta o crescimento craniofacial? Uma revisão sistemática de estudos com animais

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ABSTRACT
Sexual hormonal disturbances in humans alter the growth bone. Suppression testosterone is performed in animals for evaluated their effects on craniofacial complex. The aim of this study is to investigate, through of a systematic review from animal studies, the effects of testosterone suppression on the craniofacial complex development. Seven databases, including Open Grey literature, were searched since inception to March 01, 2021, following strategy MEDLINE for terms conducted the search. The study design PICOS was used to establish the eligibility criteria: P - Animals; I - Suppression of testosterone production; C - Animals with normal levels of testosterone; O - Effect in craniofacial growth/development; S - In vivo studies. Relevant data were collected and inserted in characteristics of studies table. Risk of bias was assessed using SYRCLE's risk of bias tool. Ten studies were included in the systematic review. Two were classified with low risk of bias and eight with unclear. The mandible in experiment group was significantly smaller than control group. The trabecular bone mineral density of the mandible was decrease after testosterone suppression. There was an increase in the number of osteoclasts in the experimental groups. All cephalometric measurements of the maxilla, except in one study, were reduced in orchiectomized rats. The expression of androgen receptor was significantly reduced in head condyle of the experimental group. Testosterone suppression decreases the growth of craniofacial complex bones through imbalance of the bone turnover due to the increase in the number of osteoclasts.

Keywords: Testosterone suppression, craniofacial growth, orchiectomy, mandible, maxilla

RESUMO
Distúrbios hormonais podem alterar o crescimento ósseo de humanos. A supressão de testosterona tem sido realizada em animais para avaliar o impacto sobre o crescimento craniofacial. O objetivo deste estudo foi investigar, através de uma revisão sistemática de estudos com animais, o imacto da supressão de testosterona sobre o complex craniofacial. Sete base de dados, incluindo literatura cinzenta, foram acessadas em 1º de Março de 2021 para a busca seguindo a estratégia MEDLINE. PICOS foi utilizado para estabeler o critério de eligibilidade: P – Animais; I – Supressão da produção de testosterona; C – Animais com níveis normais de testosterona; O – Efeitos sobre o crescimento craniofacial; S – Estudos *in vivo*. Dados relevantes foram coletados e inseridos em uma tabela de características dos estudos. Risco de viés foi avaliado pela ferramenta SYRCLE. Dez estudos foram incluidos na revisão. Dois foram classificados como baixo risco de viés e oito como inconclusivo. A mandíbula no grupo experimental menor em comparação ao grupo controle em todos os estudos. A densidade mineral óssea trabecular da mandíbula diminui após a supressão de testosterona. Houve um aumento do número de osteoclastos nos grupos experimentais. As medidas cefalométricas da maxilla foram menores no grupo experimental. A expressão de receptor de andrógeno foi menor na cabeça do côndilo no grupo experimental. A supressão de testosterona diminui o

crescimento craniofacial através do desbalanceamento do turnover osseo devido ao aumento do número de osteoclastos.

Palavras-chave:Supressão de Testosterona, crescimento craniofacial, orquiectomia, maxila, mandíbula.

1 INTRODUCTION

Sexual hormones – testosterone and estrogen – have a fundamental role in craniofacial growth (Fujita et al., 2006). In the past few decades, the authors reported that sexual hormones stimulate directly or indirectly the differentiation of osteoclasts and osteoblasts (Eriksen et al., 1988; Mizuno et al., 1994; Bellido et al., 1995) through androgen and estrogen receptors present in the structures involved in the process of periosteal, sutural, membranous and cartilaginous growth (Fujita et al., 2004; Verdonck et al., 1998 “A” and “B”).

Environmental influences and congenital factors affect the levels of sexual hormones, which harms the development of craniofacial complex (Cray Jr et al., 2011; Roosenboom et al., 2018). Studies that suppressed steroid hormones levels in animals have reported inhibition of the craniofacial complex development (Fujita et al., 2004; Fujita et al., 2006; Verdonck et al., 1998“A” and “B”; Noda et al., 1994). However, the testosterone suppression (TS) affects the craniofacial growth in higher proportions than the suppression of the estrogen (Fujita et al., 2004).

Some cephalometric measurements of the maxilla, and especially mandible, were significantly lower when testosterone was suppressed in male rats. Besides, the craniofacial bones in castrated rats at birth were smaller than those castrated rats at puberty (Duncker et al., 1988; Verdonck et al, 1998 “A”). Trabecular (Tr) and Condylar (Ct) volume and density and masticatory muscles were insufficiently studied, despite being structures with high sensitivity to testosterone (Noda et al., 1994).

The effects of exogenous testosterone were also studied. In the medical field, testosterone replacement is well described in the literature as effective in stature growth, muscle mass gain, in the treatment of osteoporosis and Turner’s syndrome (Noda et al., 1994). However, the sensitivity of androgen receptors in the craniofacial complex is different from other sites in the body (Verdonck et al., 1998 “B”). Studies in animals demonstrated that the testosterone replacement cause effects in different proportions in cartilaginous, endochondral and sutural craniofacial growth structures, resulting in a disproportionate growth of the face (Zingeser and Phoenix, 1978; Moutier et al., 1992;

Noda et al., 1994; Verdonck et al., 1998 “A” and “B”; Fujita et al., 2004; Fujita et al., 2006).

Thus, through a systematic review from animal studies, the aim of this study is to investigate the effects of TS on the craniofacial complex growth and development.

2 MATERIAL AND METHODS

Protocol and registration

This systematic review was developed based on guidelines of The Cochrane Handbook for Systematic Reviews of Interventions and the PRISMA statement. Subsequently, the study was registered in the PROSPERO database (CRD42019137882).

Eligibility criteria

Animals’ studies, which evaluate the testosterone suppression effects in craniofacial growth, were included. The study design PICOS was used to establish the eligibility criteria (table 1).

Table 1. Eligibility criteria for the present systematic review.

Domain	Inclusion Criteria	Exclusion Criteria
Participants	▪ Animals with suppression of testosterone during puberty.	▪ Humans with disturbance of testosterone ▪ Animals *knock out*
Interventions	▪ Suppression of testosterone production.	▪ Simultaneous administration of substances not considered in the present review.
Comparisons	▪ Animals with normal levels of testosterone.	
Outcomes	▪ Effect in craniofacial growth.	▪ Qualitative assessments regarding the rate of craniofacial growth.
Study design	▪ *In vivo* studies ▪ *Mammals studies*	▪ Non-comparative studies. ▪ *In vitro* or *ex-vivo* studies. ▪ Reviews, systematic reviews and meta-analyses. ▪ Less than 4 subjects per group analysed.

There were no restrictions on publication dates, sample sizes, language and statistical analyses. Unpublished manuscripts, reviews, systematic reviews, meta-analyses, and book chapters were excluded.

Information sources and search strategy

Seven databases (PubMed, Web of Science, Scopus, Cochrane, Embase and ScienceDirect), including grey literature (ProQuest), were searched since inception to March 01, 2021. The strategy MEDLINE was used and the following terms conducted

the search: "Testosterone*"[Mesh] OR "Androgens*"[Mesh] AND "Skull"[Mesh] OR "Jaw*"[Mesh] OR "Mandible*"[Mesh] OR "Maxilla*"[Mesh] OR "Facial Bones"[Mesh] OR "Palate, Hard"[Mesh] OR "Palate*"[Mesh] AND "Growth and Development"[Mesh] OR "Growth*"[Mesh] AND "Animals*"[Mesh] OR "Animal Experimentation"[Mesh] OR "Animals, Laboratory"[Mesh]. The details of search strategy are showed in table 2.

Study selection

The review was performed by two authors (C.L.B.R. and D.S.B.O.) who evaluated the titles and abstracts of the articles independently in each database. The full article was obtained when they were eligible or potentially eligible or when the title or abstract had insufficient information for the evaluation.

Table 2. Strategy for database search (up to March 01th, 2021).

Database [2021 01 03]	Search strategy	Hits
PubMed	("Testosterone*"[Mesh] OR "Androgens*"[Mesh]) AND ("Skull"[Mesh] OR "Jaw*"[Mesh] OR "Mandible*"[Mesh] OR "Maxilla*"[Mesh] OR "Facial Bones"[Mesh] OR "Palate, Hard"[Mesh] OR "Palate*"[Mesh]) AND ("Growth and Development"[Mesh] OR "Growth*"[Mesh]) AND ("Animals*"[Mesh] OR "Animal Experimentation"[Mesh] OR "Animals, Laboratory"[Mesh])	**48**
Cochrane Central Register of Controlled Trials	#1 MeSH descriptor: [Testosterone] explode all trees #2 MeSH descriptor: [Androgens] explode all trees #3 MeSH descriptor: [Skull] explode all trees #4 MeSH descriptor: [Jaw] explode all trees #5 MeSH descriptor: [Mandible] explode all trees #6 MeSH descriptor: [Maxilla] explode all trees #7 MeSH descriptor: [Facial Bones] explode all trees #8 MeSH descriptor: [Palate] explode all trees #9 MeSH descriptor: [Palate, Hard] explode all trees #10 MeSH descriptor: [Growth] explode all trees #11 MeSH descriptor: [Growth and Development] explode all trees #12 MeSH descriptor: [Animation] explode all trees #13 MeSH descriptor: [Animal Experimentation] explode all trees #14 MeSH descriptor: [Animals, Laboratory] explode all trees #15 #1 or #2 #16 {or #3-#9} #17 #10 or #11 #18 {or #12-#14} #19 {and #15-#19}	**0**
Embase	TITLE-ABS-KEY ("Testosterone*" OR "Androgens*") AND TITLE-ABS-KEY ("Skull" OR "Jaw*" OR "Mandible*" OR "Maxilla*" OR "Facial Bones" OR "Palate, Hard" OR "Palate*") AND TITLE-ABS-KEY ("Growth and Development" OR "Growth*") AND TITLE-ABS-KEY ("Animals*" OR "Animal Experimentation" OR "Animals, Laboratory")	**154**
Scopus	TITLE-ABS-KEY ("Testosterone*" OR "Androgens*") AND TITLE-ABS-KEY ("Skull" OR "Jaw*" OR "Mandible*" OR "Maxilla*" OR "Facial Bones" OR "Palate, Hard" OR "Palate*") AND TITLE-ABS-KEY ("Growth and Development" OR "Growth*") AND TITLE-ABS-KEY ("Animals*" OR "Animal Experimentation" OR "Animals, Laboratory")	**39**

ScienceDirect	title-abs-key(Testosterone OR Androgens) AND title-abs-key(Skull OR Jaw OR Mandible OR Maxilla OR "Facial Bones") AND title-abs-key(Growth) AND title-abs-key(Animals)	**17**
Web of Science™	TS=("Testosterone*" OR "Androgens*") AND TS=("Skull" OR "Jaw*" OR "Mandible*" OR "Maxilla*" OR "Facial Bones" OR "Palate, Hard" OR "Palate*") AND TS=("Growth and Development" OR "Growth*") AND TS=("Animals*" OR "Animal Experimentation" OR "Animals, Laboratory")	**5**
ProQuest	ALL(Testosterone* OR Androgens*) AND ALL(Skull OR Jaw* OR Mandible* OR Maxilla* OR Facial Bones OR "Palate, Hard" OR Palate*) AND ALL("Growth and Development" OR Growth*) AND ALL(Animals* OR "Animal Experimentation" OR "Animals, Laboratory")	**4**

Disagreements on eligibility were resolved by consensus, and when differences still persisted, a third reviewer was consulted for final decision.

Data collection and data items

The same two authors independently carry out data extraction. General information and specifics predetermined characteristics was collected: authors, year, animal model, treatment, sample size, age of treatment, craniofacial measurement method, effect on maxilla, effect on mandible, author's conclusion and additional information.

Risk of bias in individual studies

The risk of bias in individual studies was performed by two authors (C.L.B.R. and D.S.B.O.) independently with SYRCLE's risk of bias tool (Hooijmans et al., 2014). Disagreements were resolved by discussion or consultation with the third author (E.C.K.). The summary risk of bias within a study was assessed according to Higgins and Green (2019).

Quantitative data synthesis was not carried out due to the heterogeneity of the studies.

Risk of bias across studies and additional analyses

Not have a sufficient number of studies for analyses of risk of bias across studies and additional analyses.

3 RESULTS

Study selection

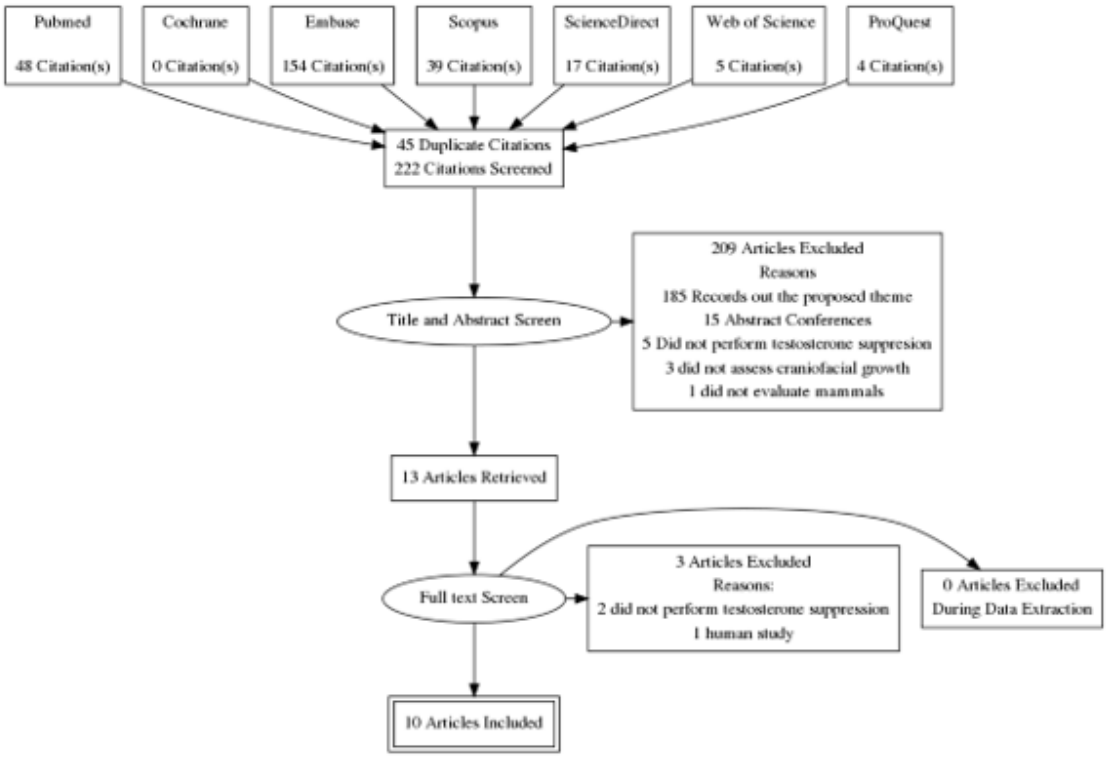
All steps for study selection are described in the diagram shown in figure 1. The authors (C.L.B.R. and D.S.B.O.) identified in databases 267 references. We excluded 45

duplicate articles and 209 more based on the title and abstract. The complete text of 13 eligible articles was obtained.

Three articles were excluded for the following reasons: studies involving humans (n = 1) and did not perform testosterone suppresion (n = 2). Finally, 10 full-articles were included in the systematic review.

Study characteristics

Figure 1: Diagram flow of PRISMA



The summary of the characteristics of the studies are presented in table 3. Most of the studies used mice and rats as animal model. Only Wang et al. (2016) used monkey species Macaca mulata for the experiment. For TS, the all the studies performed orchiectomy (ORX). ORX consists of the surgical removal of the testicles. Verdonck et al. 1998 “B” also performed chemical castration beyond the ORX. TS was performed at different time periods in each study. In rodents, the suppression ranged from after birth to 60 days of age. The suppression in monkeys ranged from 3 months to 7 years. The analysis also occurred in different time periods, varying between 2 for 16 weeks after surgery. For craniofacial measurement, 4 studies used linear measurement through cephalometric analysis with lateral cephalogram (X-ray). Fujita et al. (1998, 2001 “A”

and 2001 “B”) perform histomorphometry analysis. In 2006, besides of histomorphometry analysis, Fujita et al. performed also quantitative computed tomography. Wang et al. (2016) also used quantitative computed tomography, but they complemented with 3D X-ray. Márquez Hernández et al. (2011) was the only that performed immunohistochemical analysis.

Risk of bias within studies

Table 3. Studies results

Author/ year	Animal model	Treatment	Sample size	Age of the treatment	Craniofacial measurement method
Verdonck (1998) "A"	Wistar rats	Orchiectomy (ORX) Treated with 1.5 m Silastic Tube with testosterone (23µg/cm) implanted subcutaneously from 2 days.	1) 18 ORX implanted 2) 17 ORX 3) 15 controls	ORX After Birth Implant 57 days old.	Lateral cephalograms (X-ray) - Cephalometric analysis at day 70 and day 110.
Verdonck (1998) "B"	Wistar rats	Orchiectomy (ORX)	1) 52 ORX 2) 45 controls	ORX 4h after birth	Lateral cephalograms (X-ray) - Cephalometric analysis from day 20 until day 70.
		Chemical Castration (CC) with injection intramuscularly of 10 µ Triptorelin (IIT)	1) 20 CC in 25 days old 2) 20 CC in 25 and 45 days old 3) 24 controls.	1) CC in 25 days old 2) 25. and 45 days old.	Lateral cephalograms (X-ray) - Cephalometric analysis from day 30 to day 110
Fujita T. (1998)	C57BL/6J mice	Orchiectomy (ORX)	1) 20 experimental ORX 2) 20 control	60 days old	Bone histomorphometry analysis in the subchondral area of the condyle of mice sacrificed in 2, 4, 8 and 12 weeks after surgery.
Fujita T. (2001) "A"	C57BL/6J mice	Orchiectomy (ORX)	1) 5 experimental ORX 2) 5 control	60 days old	Bone histomorphometry analysis in the subchondral area of the condyle four weeks after surgery.
Fujita T. (2001) "B"	C57BL/6J mice	Orchiectomy (ORX)	1) 5 experimental ORX 2) 5 control	60 days old	Bone histomorphometry analysis of the condyle eight weeks after surgery.
Lerouxel et al (2004)	Wistar rats	Orchiectomy (ORX)	1) 24 experimental ORX 2) 24 control SHAM	After Birth	Dual energy X-ray absorptiometry (DXA) and high-resolution X-ray in 2, 4, 8 or 16 weeks after surgery.
Fujita T. (2004)	C57BL/6J mice	Orchiectomy (ORX)	1) 15 experimental ORX 2) 10 control	5 days old	Lateral cephalograms (X-ray) - Cephalometric analysis four weeks after surgery.
Fujita T. (2006)	C57BL/6J mice	Orchiectomy (ORX)	1) 20 experimental ORX 2) 5 control	5 days old	Histomorphometry analysis of trabecular (Tr) and cortical (Ct) bone mineral density (BMD) by peripheral quantitative computed tomography (pQCT) of mandible eight weeks after surgery.
Márquez-Hernández et al. (2011)	C57BL/6J mice	Orchiectomy (ORX) Ovariectomy (OVX)	1) 10 experimental ORX 2) 10 control	5 days old	Immunohistochemical analysis (ERα, ERβ, and AR) and Cephalometric analysis of only three measurements one week after surgery.
Wang Qian (2016)	Rhesus Macaques	Orchiectomy (ORX)	1) 4 experimental ORX (CM) 2) 11 control in prime age 3) 8 control in old age	In experimental group: 3 months to 7 years	3D X-ray and cone beam computed tomography

Only Verdonck et al. (1998 "A") and Márquez Hernández et al. (2011) were considered which "low" risk of bias. The other studies were considered "unclear" according to the SYRCLE's risk of bias tool. The details of the results for each article are described in Table 4.

Results of individual studies

The mandible in experiment group of the studies that used linear measurements was significantly smaller than control group. Only Verdonck et al. (1998 "B") not observed a significant difference between groups. Fujita et al. (1998, 2001 "A" and "B") described a decrease in Tr bone volume. Lerouxel et al. (2004) reported a reduction of Tr bone mineral density (BMD) in ORX mice. Besides reduction of TR-BMD, Fujita et al. (2006) also reported a reduction of CT-BMD. In addition, the histomorphometry analysis pointed out an increase of the osteoclast cells in the condyle. Wang et al. (2016) analyzed that the distance between the two rami of monkeys ORX was relatively narrower than in intact males leading to a comparatively narrower and longer face. Márques-Hernández et al. (2011) observed that the expression of AR was significantly reduced in head condyle of the experimental group. Only Fujita et al. (2004) evaluated the effects of the testosterone suppression in maxilla. The authors observed that all, except one, cephalometric measurements were significantly smaller in the operated group.

Risk of bias across studies and additional analyses

Due to the variability in methodology in each study, it was not possible to conduct analyzes for publication bias or subgroup analyzes.

4 DISCUSSION

Testosterone is the predominant androgen in men, for this reason, in the present systematic review, we decided to evaluate the existing evidence of the effects of testosterone suppression on the craniofacial complex in animals. About 95% of the secreted testosterone is produced in the testicles and the rest is produced by the adrenal glands (Vanderschueren et al., 2014). Trough of the Gonadotropin-Releasing Hormone (GnRH) regulated by hypothalamus, the pituitary gland stimulates the release of luteinizing hormone (LH), which active directly the testosterone production by Leydig cells in testicles (Mohamad et al., 2016). Testosterone acts

Table 4: Summary of the risk of bias assessment

Study	Signalling questions										
	1	2	3	4	5	6	7	8	9	10	Summary
Verdonck et al. (1998) “A”.	Low	Low	Unclear	Low	Unclear	Low	Unclear	Unclear	Low	Low	Low
Verdonck et al. (1998) “B”.	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Fujita et al. (1998).	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Fujita et al. (2001) “A”.	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Unclear
Fujita et al. (2001) “B”.	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Lerouxel et al. (2004).	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Fujita et al. (2004).	Low	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Fujita et al. (2006).	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Unclear
Márquez Hernandez et al. (2011).	Low	Low	Unclear	Unclear	Low	Low	Low	Unclear	Low	Low	Low
Wang et al. (2016).	High	High	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	High	Unclear

Notes: 1: Was the allocation sequence adequately generated and applied? 2: Were the groups similar at baseline or were they adjusted for confounders in the analysis? 3: Was the allocation adequately concealed? 4: Were the animals randomly housed during the experiment? 5: Were the caregivers and investigators blinded to the intervention that each animal received? 6: Were animals selected at random for outcome assessment? 7: Was the outcome assessor blinded? 8: Were incomplete outcome data adequately addressed? 9: Are reports of the study free of selective outcome reporting? 10: Was the study apparently free of other problems that could result in high risk of bias?

Table **5**: Authors conclusion

Author/ year	Effect on Maxilla	Effect on Mandible	Author’s conclusion	Additional information
A. Verdonck (1998) A	Not reported	The mandible were significantly lower in castrated group. Significant difference between the testosterone implanted and the control groups and not between the testosterone-implanted and the castrated groups were observed	Craniofacial variables of sutural growth mechanisms are more affected by testosterone replacement than structures with an endochondral growth mechanism.	The testosterone released from the implant did not enhance these variables significantly. This could be explained because the timing of the implantation may be late or the effects on growth induced by exogenous testosterone might differ from those in the normal growth in rats.
A. Verdonck (1998) B	Not Reported	The overall length of the mandible was not affected by neonatal surgical or by prepubertal chemical castration.	The structures showing periosteal growth as well as structures showing cartilaginous growth were affected. The growth-suppression effect was larger for the neonatally than for the prepubertally castrated rats.	How the condyle embodies the cartilaginous growth site of the mandible and an effect could be demonstrated in the cranial base, which also grows by a cartilaginous mechanism (synchondrosis), an effect was expected.

Fujita T (1998)	Not reported	TRAP-positive cells increased most substantially 8 weeks after surgery. Decrease in the trabecular bone volume was also observed during this phase.	ORX influences the remodeling of the condylar head	The present study emphasizes different timing in the influences of androgen on the expression of osteoclasts. Aromatase alters androgen.
Fujita T. (2001) A	Not reported	The condylar heads of the ORX mice exhibited a significantly greater number of osteoblasts than those controls. The trabecular bone volume of the condylar head decreased after ORX.	Decrease in the trabecular bone volume due to the lack of androgen originates in more excessive bone resorption than bone formation.	This study suggests a possibility than bone formation was promoted by the lack androgen, if an impediment of bone formation exists. It is thus anticipated in future studies to elucidate an unknown factor involved in decrease in the trabecular (TR) volume.
Fujita T. (2001) B	Not Reported	Differences in the thickness between the ORX and control groups were not significant. The ORX mice exhibited a decrease in TR bone volume, significantly different from the controls. No significant differences in the breadth of the condylar head were found between the ORX mice and the controls.	The sex hormones influence the morphogenesis of condyle in terms of the breadth.	Decrease of TR bone volume by ORX is due to increase of osteoclasts, which is further pertinent to inhibition of endochondral ossification. Condylar breadth may be increased by multiplication of condylar cartilage layer because cartilage layer never alter trabecular bone.
Lerouxel et al (2004)	Not Reported	Bone Mineral Density (BMD) decreased in the ORX. Alveolar bone loss was often visible on X-rays to the naked eye in the ORX groups, especially at 16 weeks.	Testosterone deprivation induces alveolar bone loss in the male rat. The mandibular bone loss was appreciated by BMD measurements with dual energy absorptiometry at 16 weeks. Alterations in the architectural arrangement of the alveolar bone can be assessed by texture analysis of radiographs as early as 2 weeks post-ORX.	Mandibular alveolar bone appears an interesting site for the study of bone loss induced by ORX in the adult male rat. It is likely that interference with growth is avoided because the mandible does not possess growth cartilage at the difference of long bones that are usually studied
Fujita T. (2004)	All measurements of the maxilla, except one, were significantly smaller in ORX group.	Six measurements of the mandibula were significantly smaller than in the sham-operated group.	The suppression of testosterone secretion in the growth phase might inhibit craniofacial growth and result in poor craniofacial development in the growth phase.	The growth of the maxilla, that was mainly determined by sutural growth, was inhibited in the experimental groups, as was that of the mandible, determined by membranous and cartilaginous growth. Testosterone is a critical factor not only for membranous and cartilaginous growth but also for sutural growth.

Fujita T. (2006)	Not Reported	Tr-BMD and Cortical (CT)-BMD of the mandible were significantly lower in the ORX mice. TR bone volume of the condyle, was significantly less in the experimental mice. The total thickness of the articular cartilage layers was significantly greater in the ORX mice.	Mandibular growth is inhibited by testosterone and the relevant internal structures changed. These findings indicate that sex hormones are one of the key determinants of mandibular growth and development immediately after birth.	Sex hormone deficiency may disturb endochondral ossification. Testosterone alter condylar remodeling, leading to degenerative changes in the temporomandibular joint.
Márquez-Hernández et al. (2011) A	Not Reported.	In the ORX group, the AR expression was significantly reduced when compared with the corresponding control group,.	Immediately after birth, testosterone deficiency reduces the expression of sex hormone receptors on chondrocytes in and mandibular cartilage, leading to growth disturbance.	Androgen is able to prevent bone loss in OVX rodents. It is assumed that the mandibular condyle is affected more than the mandibular body since condylar height is mainly controlled by endochondral bone formation.
Wang Qian et al (2016)	Not Reported.	Experimental group (CM)had significantly longer mandibles than control in prime age (MPA), but not control in old age (MOA), while MOA had remarkably longer mandibles than MPA. Not have significant difference among the three male groups in height and breadth. In the CM, the distance between the two rami was relatively narrower than in intact males leading to a comparatively narrower and longer face. The castrated males had thinner and lower symphyses compared with both intact males groups. In physiological corpus, although there was no significant difference. the castrated males had relatively thinner and lower corpora compared with two intact males groups, The castrated males had taller yet narrower rami compared with two intact male groups.	In the ORX, the distance between the two rami were narrower than in intact males indicating a relatively narrower and longer face; both the mandibular body and ramus had thinner cortical bone leading to less total bone mass. Dental health professionals may want to consider asking their male patients about their use of hormone (testosterone) replacement therapy as part of an overall medical history questionnaire.	In CM, the mandibles were generally longer, taller and narrower than in intact males, while the teeth were slenderer and the TMJs were smaller. The teeth cusps/crowns were lower and less robust. The longer mandibles, in both absolute and relative size, indicate a longer and narrower face in castrates.

in cells after their biotransformation into 5 α-diidrotestosterone, formula of greater affinity for androgen receptors (AR) (LaBrie et al., 2009). The main bone cells, osteoblast, osteoclast and osteocytes have AR and estrogen receptor α and β (ERα e ERβ). Growth hormone (GH) and insulin-like growth factor I (IGF-1) are also involved in bone growth and are indirectly associated with testosterone (Venken et al., 2009). Any dysfunction in hormones level would entail to deregulation of the bone remodeling process (Mohamad et al., 2016).

Androgen deficiency speeds up the bone turnover and causes an imbalance between bone reabsorption and remodeling (Lerouxel et al., 2004; Machado et al., 2021). This process occurs after a proliferation of the immature osteoblasts which excrete Receptor activator of nuclear factor kappa-B ligand, RANKL. This mediator protein stimulates differentiation and proliferation of the osteoclasts, resulting in bone loss (Clarke and Khosla, 2009). This process in the mandible is described by Fujita et al. (2001 "A"), which identified an increase of the proliferation of osteoblasts and osteoclasts in the head of the mandibular condyle in the ORX group.

Despite the advances, the molecular mechanism between AR and bone growth are still inconclusive. Some studies suggest that the receptor is directly involved in the regulation of the number of osteoclasts trough of signalizations still unknow (Wu et al., 2019 "A"). The receptor also would be responsible for regulation of osteoblasts apoptosis (Kousteni et al., 2002). The mass and mineral density of the Trabecular and Cortical bone apparently are sensitive to homeostatic maintenance of the AR concomitantly with the serological levels of the testosterone available in the blood (Wu et al., 2019 "B"). However, Manolagas et al., 2013, disagree about of the AR influence under the bone Cortical mass, reporting not have differences after TS (). Venken et al., 2009, supports the hypothesis that the growth of the trabecular bone does not suffer or suffer minimally influences of the GH and IGF-1. Then, testosterone suppression directly affects the trabecular bone. As cortical bone suffer influences of the GH and IGF-1 hormones, future studies should clarify whether testosterone plays a role on cortical bone or whether TS causes a rebound effect on the hormones GH and IGF-1 to reward suppressed testosterone.

Two types of bone growth exist in the craniofacial complex: The intramembranous type, in the most part, occur in the maxilla's suture; and the endochondral type occur in the condyle and synchondroses mandibular (Jing et al., 2016). Verdonck et al. 1998 "A" and Fujita et al. 2001 "B" agree to report that the

intramembranous growth it seems be more affected for testosterone suppression (TS) than endochondral growth. The authors observed significative alteration in the BMD in the Tr bone of the mandible, but no reported difference in the Ct bone when evaluating the condyle thickness, where occurs the endochondral growth almost exclusively.

However, Fujita et al. in their study in 2006 observed significant difference both in endochondral growth and intramembranous growth in the mandible. This study also evaluated the decrease of the Tr-BMD and Cr-BMD, which further supports the hypothesis of which TS affected both bone growth types. Unfortunately, Márquez Hernandez et al. (2011) evaluated the immunostaining of the AR only in the condylar site and did not establish parameters with the mandible body, which makes it difficult to conclude the question about the different sensitives of the sites of mandible growth.

Orthopedics studies associated low levels of testosterone in adults with low body mineral density (BMD) (Schmidt et al., 2009). The low BMD is one of the factors related to high risk of bone fractures, mobility and dental loss (Singh et al., 2011; Ji et al., 2016). A cohort study performed with 2.908 Swedish elderly demonstrated the data of Lerouxel et al. (2004) and Fujita et al. (2006), of which TS are associated with low BMD and Cr bone size. The same relationship was not observed with estrogen levels.

The methods used for cephalometric analysis in the mandible varied between studies. Verdonck et al. (1998 “A”, “B”) traced the radiographs with the purpose of evaluating different growth sites. Márquez Hernandez et al. (2011) traced only 3 measurements for evaluated the endochondral growth in the mandible. Fujita et al. 2004 proposed similar measurements to that applied in human cephalometry, besides drawing the measurements also in the maxilla. Only one distance in the maxilla were not affected for the ORX, instead of the mandible, in which only 6 was affected. Thus, it is possible to affirm that growth sutural is more sensitive to TS than endochondral growth.

Regarding the studies included in this review, the results are consistent and are in agreement with evidence of studies in basic area of endocrinology and orthopedics. The authors report more coherent and comprehensive data as different methods of analysis are applied in the same study. Statistical analyzes of all articles were properly conducted and enlightening.

It is necessary to infer about some factors that imply in inconclusive results between studies. Some studies not follow the recommended period to perform prepubertal ORX, which is between 21 into 28 days for mice (Kohn e Clifford, 2002; Guénet et al., 2015) and between 21 into 35 for rats (Otto et al., 2015). Besides that, ORX soon after

birth is not recommended because of the neonatal mortality rate. It is recommended to perform the ORX from 5 days of life in mice and rats to evaluate the effects of TS before puberty (Márquez Hernández et al., 2011).

Many articles do not describe the quantitative data of linear measurements. Detailed information is necessary to carry out an accurate assessment of each measure and establish parameters for future studies. The absence of photographs in some studies prevents the deepening of the results and discussions, mainly of histological sections. In addition, it is of utmost importance to describe the serological levels of testosterone or in the articles, both to confirm the efficacy of ORX and to establish statistical differences related to the amount of circulating testosterone, which was neglected by most studies.

About the biases described through the SYRCLE scale, few authors did not perform adequate randomization and blindness of the investigators or even reported in the articles. Randomization and blinding increase the reliability of the studies because researchers are not induced to report convenient results.

The effects of TS on the maxilla should be investigated in future research. Future studies need to be delineated with adequate randomization and blindness to reduce the risk of bias. Methodologies of modern analyzes, such as microtomography and immunohistochemical, can be applied to promote more consistent discussions, mainly to clarify the different functions that the androgen has in the Tr and Ct bones. Finally, the biomolecular mechanisms involved in this process need to be investigated to find the AR-mediated signals for maintaining the number of osteoclasts in the bone.

5 CONCLUSIONS

From the data collected and the characteristics of each study described in this systematic review, it can be concluded that TS decreases the growth of craniofacial complex bones through imbalance of the bone turnover due to the increase in the number of osteoclasts. These factors should be considered by the professional orthodontist to avoid possible disorders in the proposed treatment.

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