ANDRÉ DE SOUZA

ESTÍMULOS ENDÓCRINO PODEM REGULAR A DINÂMICA FIBROBLASTOS/MIOFIBROBLASTOS DURANTE A CICATRIZAÇÃO DE FERIDAS CUTÂNEAS? UMA REVISÃO SISTEMÁTICA DE MODELOS PRÉ-CLÍNICOS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

Orientadora: Reggiani Vilela Gonçalves

Coorientador: Rômulo Dias Novaes

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Assentimento:

André de Souza
Autor

Reggiani Vilela Gonçalves
Orientadora

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RESUMO

SOUZA, André de, M.Sc., Universidade Federal de Viçosa, novembro de 2022. **Estímulos endócrinos podem regular a dinâmica fibroblastos/miofibroblastos durante a cicatrização de feridas cutâneas? Uma revisão sistemática de modelos pré-clínicos**. Orientadora: Reggiani Vilela Gonçalves. Coorientador: Rômulo Dias Novaes.

Fibroblastos e miofibroblastos são células importantes envolvidas na contração da ferida, bem como na síntese de importantes componentes da matriz extracelular (MEC). No entanto, os principais mecanismos envolvidos na diferenciação de miofibroblastos após exposição a hormônios são pouco compreendidos. Portanto, o objetivo deste estudo foi revisar sistematicamente a ação da estimulação endócrina sobre os miofibroblastos durante o processo de cicatrização de feridas cutâneas por meio de análises in vitro e in vivo. Além disso, realizamos uma revisão crítica da qualidade metodológica desses estudos usando o risco de viés SYRCLES. As bases de dados pesquisadas foram PubMed/Medline, Scopus e Web of Science, e apenas estudos originais foram analisados de acordo com as diretrizes PRISMA. Estudos in vivo mostraram que hormônios lipídicos como testosterona, estrogênio e 17-b estradiol estimulam a diferenciação de fibroblastos em miofibroblastos enquanto o DHT prejudica esse processo aumentando os níveis de citocinas pró-inflamatórias e bloqueando a expressão de TGF-β pela via de sinalização do Receptor Androgênico (AR)-Smad3. O hormônio peptídico GH, além de promover o aumento dos níveis de IGF-1, extensão da fase inflamatória, inibe a diferenciação de fibroblastos em miofibroblastos induzida por TGF-β, enquanto o peptídeo semelhante ao glucagon (GLP-1), o hormônio GHRH e o corticoide sintético Dexametasona (DX) reduzem a expressão de marcadores inflamatórios como COX-2 e promove um aumento na expressão de α-SMA, promovendo a contração da ferida. Nas análises in vitro, o hormônio peptídico GHRH promoveu um aumento na marcação de α-SMA, enquanto o GH reduziu esses marcadores, corroborando as análises in vivo. As principais vias estudadas foram TGF-β/Smad, MAPk e vias de bloqueio de mediadores como NFKb. Além disso, observou-se que a dose varia de 1 a 10mg/kg de estrogênio e 17-β estradiol, assim como 2mg de Dexametasona aumentam a coloração para miofibroblastos, e em GLP-1 e GHRH, doses de 3mg/kg e 100nM, respectivamente.

Os estudos apresentam dados heterogêneos e limitações metodológicas, porém, a semelhança entre alguns estudos permitiu a realização de uma metanálise considerando o modelo experimental, tempo de tratamento e doses, levando a resultados consistentes e maior confiabilidade. Este estudo está registrado na plataforma PROSPERO (CRD42021264735).

Palavras-chave: Regulação endócrina. Hormônios. Miofibroblastos. Cicatrização.

ABSTRACT

SOUZA, André de, M.Sc., Universidade Federal de Viçosa, november, 2022. **Could endocrine stimuli regulate fibroblasts/myofibroblasts dynamics during skin wound healing? A systematic review of preclinical models.** Adviser: Reggiani Vilela Gonçalves. Co-adviser: Rômulo Dias Novaes.

Fibroblasts and myofibroblasts are important cells involved in wound contraction, as well as in the synthesis of important components of the extracellular matrix (ECM). However, the main mechanisms involved in the differentiation of myofibroblasts after exposure to hormones are poorly understood. Therefore, the aim of this study was to systematically review the action of endocrine stimulation on myofibroblasts during the cutaneous wound healing process using in vitro and in vivo analyses. In addition, we performed a critical review of the methodological quality of these studies using the SYRCLES risk of bias. The databases searched were PubMed/Medline, Scopus and Web of Science, and only original studies were analyzed according to the PRISMA guidelines. In vivo studies have shown that lipid hormones such as testosterone, estrogen and 17-b estradiol stimulate myofibroblast differentiation while DHT impairs this process by increasing the level of pro-inflammatory cytokines and blocking the expression of TGF-β through the signaling pathway. of the Androgen Receptor (AR)-Smad3. The peptide hormone GH, in addition to promoting the increase of the IGF-1, extension of the inflammatory phase, inhibits the differentiation of fibroblasts into myofibroblasts induced by TGF-β, while the glucagon-like peptide (GLP-1) and the hormone GHRH and dexamethasone (DX) reduce the expression of inflammatory markers such as COX-2 and increase the expression of α-SMA, promoting wound contraction. In in vitro analyses, the peptide hormone GHRH promoted an increase in α-SMA labeling, while GH reduced these markers, corroborating the in vivo analyses. The main pathways studied were TGF-β/Smad, MAPk and blockade of pro-inflammatory pathways such as NFKb. Furthermore, it was observed that the dose varies from 1 to 10mg/k of estrogen and 17-β estradiol as well as 2mg of dexamethasone increase staining for myofibroblasts and in GLP-1 and GHRH, doses of 3mg/k and 100nM respectively. The studies present heterogeneous data and have methodological limitations, however, the similarity between some studies allowed the performance of a meta-analysis considering an experimental model, treatment time

and doses, leading to consistent results and greater reliability. This study is registered on the PROSPERO platform (CRD42021264735).

Keywords: Endocrine regulation. Hormones. Myofibroblasts. Healing.

LISTA DE SIGLAS E ABREVIATURAS

AR: Androgen receptor

DHT: Dihydrotestosterone

TGF-β: Transforming growth factor beta

GH: Growth Hormone

GHRH: Growth hormone releasing hormone

COX-2: Cyclooxygenases

CYP19A1: Cytochrome P450 Family 19 Subfamily A Member 1

DX: Dexamethasone

MAPk: Mitogen-activated protein kinase

NFKb: Factor nuclear kappa B

GLP-1: Glucagon-like peptide

ECM: Extracellular matrix

α-SMA: Alpha-Smooth Muscle Actin

EMT: Epithelial-mesenchymal transition

EndoMT: endothelial-mesenchymal transition

PDGF: Platelet-derived growth factor

COL1A1: collagen type I alpha I

MMP: metalloproteinases

NO: Nitric Oxide

M2: Macrofage 2

DPP-4: dipeptidyl peptidase-4ABNT

SUMÁRIO

1. INTRODUÇÃO GERAL

1.1. Cicatrização de feridas cutâneas

A pele é um órgão que proporciona uma barreira de proteção contra o meio ambiente e da perda de água. Distúrbios cutâneos originados de diferentes modos de lesão física ou doenças, pode levar a complicações corporais, disfunções e até fatalidade (Gushiken et al., 2021). Em 2019, dados globais indicaram um total de 8.378.122 novos casos de queimaduras (Yakupu et al., 2022). Além disto, existe um aumento vertical relacionado ao número de doenças de pele e subcutâneas nos últimos anos, com 605.036.000 no ano de 2015 em comparação com 492.883.000 em 2005 (Vos et al., 2015). As consequências são grandes gastos por parte dos serviços de saúde mundiais e perda da qualidade de vida dos indivíduos exigindo maiores investimentos na geração de recursos materiais e humanos para promover um cuidado eficaz a estes pacientes (Silva et al., 2017).

A busca pela compreensão dos mecanismos celulares e moleculares envolvidos no processo de cicatrização de feridas é fundamental para o direcionamento de pesquisas para o desenvolvimento de estratégias terapêuticas. Em geral, uma ferida consiste em uma incisão, punção ou abrasão da epiderme da pele, derme e dos músculos subdérmicos e tecidos adiposos subjacentes. O reparo cutâneo envolve a ativação e liberação de diferentes células e mediadores inflamatórios que são responsáveis por criar um ambiente de homeostase celular e tecidual, e assim permitir a síntese de importantes componentes da matriz que promoverão o fechamento das lesões de pele (Lux, 2022). A cicatrização de feridas é um processo multifacetado que envolve diferentes tipos celulares, incluindo queratinócitos, e outras células epidérmicas, fibroblastos/miofibroblastos, melanócitos, células endoteliais e células nervosas periféricas (Rieger et al., 2015). O processo de cicatrização de feridas pode ser dividido em três fases principais – inflamação, proliferação e remodelação tecidual – que se sobrepõem no tempo (Gushiken et al., 2021). A primeira fase do processo de cicatrização é marcada principalmente pela migração de células do sistema imune para a área da lesão, facilitada por vasodilatação, aumento da permeabilidade vascular e liberação de substâncias quimiotáticas liberadas pelas plaquetas (Tottoli et al., 2020). Já a transição da fase inflamatória para a fase proliferativa depende da segregação de vários agentes quimiotáticos, citocinas e

fatores de crescimento que ativam elementos celulares que serão importantes na fase subsequente (Kim et al., 2019). Na fase proliferativa, os fibroblastos se diferenciam em miofibroblastos e sintetizam colágeno, glicosaminoglicanos e fibronectina, sendo responsáveis pela formação do tecido de granulação e da contração da ferida (Desmouliere et al., 2014), sendo então conhecidos como fatores-chave na reconstrução fisiológica do tecido conjuntivo após a lesão tecidual (Hinz, 2016), e por fim temos a fase de remodelação, caracterizada pela maturação do tecido formado com a substituição do colágeno tipo III por colágeno tipo I oferecendo uma maior resistência do tecido cicatricial (Altoe et al 2020).

Gabbiani et al.1971, demostraram que a contração nas bordas da ferida é ativamente promovida por fibroblastos especializados, denominados de “miofibroblastos”. Entretanto, já é conhecido que, apesar de sua importância para restaurar a integridade do tecido cicatricial, as atividades dos miofibroblastos após um determinado tempo precisam cessar, e o grande número destas células características da fase proliferativa precisa ser reduzido por apoptose para que ocorra a formação de uma cicatriz forte e com fibras colágenas organizadas. Já se sabe que a persistência da atividade dos miofibroblastos e o grande número destas células pode levar à formação de tecido hipertrófico, caracterizado por alta densidade de colágeno produzido por esta célula (Lee et al., 2019), podendo levar à formação de lesões fibróticas (Schuster et al., 2022).

A diferenciação de fibroblastos em miofibroblastos compreende diferentes processos que podem ser ativados a partir de múltiplas fontes celulares, variando com o ambiente local e o tipo de lesão, para restaurar a integridade do tecido (Schuster et al., 2022). Destas fontes podemos destacar o contato célula-célula e célula-matriz, o desenvolvimento de feixes contráteis em resposta a propriedades mecânicas da matriz extracelular (MEC) (Sheldon et al., 2021) e as citocinas liberadas localmente por células inflamatórias e residentes (Hinz and Lagares, 2020). Neste contexto, podemos destacar que estas interações mecânicas ou químicas, são responsáveis por estimular a expressão de α-SMA e consequentemente pela diferenciação de fibroblastos e formação de miofibroblastos. Vale ressaltar que a ação conjunta de fatores de crescimento como o fator de crescimento transformador beta (TGFb1), de proteínas especializadas da MEC como a variante de fibronectina (FN) ED-A FN e o microambiente mecânico promove a recuperação do tecido (Suzuki et al., 2020).

Neste contexto, os mecanismos que envolvem a diferenciação de fibroblastos em miofibroblastos, bem como as estratégias terapêuticas que ativam estes mecanismos tem se destacado no processo de investigação.

1.2. Ação hormonal

Hormônios são substâncias derivados de aminoácidos (aminas), esteroides (colesterol), glicoproteínas ou polipeptídios (proteínas), e seus efeitos são condicionados à ligação com seus respectivos receptores. Além disso, atuam como verdadeiros reguladores sistêmicos de controle homeostático, visando o equilíbrio das células, dos tecidos, órgãos e sistemas e dessa forma estabilizando funções fisiológicas (Neumann et al., 2019); e seu desequilíbrio pode afetar diferentes órgãos-alvo alterando por exemplo o reparo tecidual (Wen et al., 2022). Assim, de forma geral, os hormônios são moduladores de reações, participando de funções específicas, tais como crescimento e diferenciação celular, regulação do metabolismo, entre outros (Bereshchenko et al., 2018; Sinha et al., 2018; Troisi et al., 2018). Os efeitos hormonais são viabilizados através de receptores específicos de células-alvo que podem ser de membrana ou intracelulares. A interação hormônio/receptor promove modificações conformacionais alterando metabolismo e funções celulares especificas (Moulton, 2018; Rieger et al., 2015; Stuard et al., 2020). Além disso, estruturas análogas que se unem ao receptor podem ocasionar os mesmos efeitos (Blumenfeld, 2019; Schally et al., 2019). O número de receptores varia para cada tipo de célula, variando, portanto, o grau da resposta de cada célula à ação hormonal (Cole et al., 2019).

O processo de reparação de feridas cutâneas é profundamente influenciado pela ação de diferentes hormônios. Nos últimos anos, estímulos endócrinos tem sido investigados quanto a sua influência no processo de cicatrização de feridas cutâneas, em especial na diferenciação de fibroblastos em miofibroblastos (Caicedo and Devesa, 2019; de Almeida et al., 2016; Guo and DiPietro, 2010). Quando hormônios ou seus análogos ligam-se a seus receptores ativando diferentes vias celulares, promovem modificações no tecido cicatricial de granulação, ativando fibroblastos que passam a expressar alfa actina do músculo liso (α-SMA) e se tornam miofibroblastos (Schuster et al., 2022), estes sintetizam e depositam maior quantidade de componentes fibrilares da matriz extracelular (MEC), que são essenciais para o reparo

tecidual eficiente (Darby et al., 2014). Entretanto, um desequilíbrio endócrino pode alterar o processo de reparo tecidual por diferentes mecanismos (Gushiken et al., 2021). Assim, considerando a necessidade de compreensão destes diferentes mecanismos de ação hormonal no processo de cicatrização, neste estudo realizamos uma revisão sistemática para compreender os efeitos dos estímulos endócrinos na dinâmica dos fibroblastos/miofibroblastos durante a cicatrização da pele. Revisões sistemáticas são consideradas trabalhos de alto nível que permitem a avaliação individual dos estudos de forma cega por meio de ferramentas específicas (Page et al., 2021). Estas características levam a uma abordagem mais inclusiva, e não tendenciosa, proporcionando ao leitor uma compreensão ampla dos estudos incluídos, direcionando investigações futuras sobre a interação hormônio/miofibroblastos, e produção de componentes da MEC, além de auxiliar na investigação de novos alvos terapêuticos.

2. OBJETIVOS

2.1. Objetivo Geral:

Avaliar por meio de revisão sistemática o efeito do estímulo endócrino na dinâmica dos fibroblastos/miofibroblastos durante a cicatrização de feridas cutâneas em modelos pré-clínicos.

2.2. Objetivos específicos

- Realizar uma revisão sistemática sobre a influência da ação de hormônios na cicatrização de feridas cutâneas com ênfase na fase de diferenciação de fibroblastos em miofibroblastos utilizando análises *in vitro* e *in vivo*;
- Identificar as principais classes de hormônios bem como sua influência no processo de diferenciação de miofibroblastos durante a cicatrização de feridas cutâneas em modelo *in vitro* e in *vivo*
- Identificar as principais vias celulares e moleculares estimuladas por hormônios durante a cicatrização de feridas cutâneas;
- Identificar os ricos de viés metodológico dos estudos incluídos.

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4. ARTIGO

COULD ENDOCRINE STIMULI REGULATE FIBROBLASTS/MYOFIBROBLASTS DYNAMICS DURING SKIN WOUND HEALING? A SYSTEMATIC REVIEW OF PRECLINICAL MODELS

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ABSTRACT

The interaction of hormones and their respective nuclear receptors (NHRS) directly interfere in the tissue repair process, stimulating or inhibiting the expression of myofibroblasts. The main mechanisms involved in fibroblasts differentiation into myofibroblasts after hormones exposure are poorly understood. Therefore, we systematically reviewed the impact of endocrine stimulation on myofibroblasts during skin wound healing using in vitro and in vivo preclinical models. The methodological quality of these studies was investigated using the SYRCLE's risk of bias tool. PubMed/Medline, Scopus, and Web of Science databases were used, and only original studies were analyzed according to the PRISMA guidelines. In vivo results showed that lipid hormones such as testosterone, estrogen, and 17-β estradiol stimulated fibroblast differentiation, while hormones like dihydrotestosterone impaired this process by increasing pro-inflammatory cytokines expression, blocking TGF-β production via androgen receptor (AR)-Smad3 inhibition. Conversely, peptide hormones like growth hormone (GH) promoted the increase of the IGF-1, potentiated the inflammatory phase, inhibiting fibroblasts differentiation into myofibroblasts, as indicated by reduced alpha-smooth muscle actin (α-SMA) expression. However, there is no consense about growth hormone-releasing hormone (GHRH), which can inhibit inflammatory effectors such as COX-2 and increase α-SMA expression, promoting wound contraction. However, glucagon-like peptide (GLP-1) and dexamethasone (DX) down-regulate inflammatory effectos such as COX-2 and stimulate α-SMA expression, promoting wound closure. In general, TGF-β/Smad, MAPK, and NF-kB pathways were investigated in all studies reviewed. The results were heterogenous since different doses and times of hormones exposure were analyzed. Furthermore, the evidence indicated that 1 to 10 mg/kg estrogen and 17-β estradiol, as well as 2 mg/kg dexamethasone increase myofibroblasts immunostaining. Despite the methodological limitations, similarities between some studies (e.g., experimental model, treatment time, and doses) allowing-us develop a meta-analysis, reaveling consistent results with greater reliability. This study was registered on the PROSPERO platform (CRD42021264735).

Keywords: Endocrine regulation, Hormones, Myofibroblasts, Healing.

1. INTRODUCTION

Skin wounds are serious medical-social problems with difficult clinical approach, especially considering the marked variability of manifestations that develop throughout its evolution (Xie et al., 2013). Around 10% inpatients and 20% bedridden patients treated at home are believed to suffer from skin ulcerations (Assis de Brito et al., 2014). In a systematic review and meta-analysis of observational studies between 2010 and 2017, Martinengo et al. (2019) showed a high prevalence of chronic wounds in the general population, mainly leg injuries. This study also revealed that chronic wounds affect 2.21 in each 1000 inhabitants, mainly in European countries (65%) (Martinengo et al., 2019). Typically, these injuries ae more frequent in individuals between 50 and 80 years-old. According to the World Health Organization (WHO), it is estimated that skin wounds prevalence will increase from 12 to 22% by 2050 (LeBlanc et al., 2020). In this context, new formulations are needed to treat skin diseases and improve the quality of life in this population. Thus, understanding the pathophysiological processes and the main mechanisms involved in tissue repair, including fibroblasts differentiation, is relevant to delimit new biotecnological strategies focused on stimulating skin wound healing.

Cutaneous repair is a complex event coordinated by cells, cytokines, and growth factors. Accordingly, fibroblast are important cells that release cytokines and growth factors essential in all wound healing phases. These cells can differentiate into myofibroblasts, promoting more efficient wound closure (Sarandy et al., 2015). Thus, dermal fibroblasts proliferate and migrate during healing, activating gene expression for α-smooth muscle actin (α-SMA), which is required for myofibroblasts differentiation. Myofibroblasts were first described by Gabbiani et al. (1971) and were observed in the

granulation tissue of skin wounds in rats. Studies report that myofibroblasts can originate from different cell types, and extracellular matrix (ECM) components can influence this differentiation (Pakshir et al., 2020). Myofibroblasts show morphologically similarities with fibroblasts and smooth muscle cells, exhibiting well-distributed mitochondria, developed golgi complex, extensive rough endoplasmic reticulum cisterns, and packed fibrillar bundles similar to smooth muscle cells (Hinz, 2016). Furthermore, myofibroblasts express adhesion molecules such as ICAM and VCAM, stimulating cell adhesion and migration at the beginning of the inflammatory process. These cells also develops a effective communication process, which is mediated by autocrine and paracrine connections that allow the exchange of soluble cytokines and growth factors (Bagalad et al., 2017). In addition, environmental stress like mechanical tension of the wound, can promote the phenotype transition from fibroblast to myofibroblast (D'Urso and Kurniawan, 2020). However, the effect of intrinsic and extrinsic stimuli, including hormones exposure during wound healing remains unclear. Currently, there is evidence that skin repair is influenced by hormones that promote modifications in several cellular mechanisms, especially in fibroblasts and myofibroblasts (Levine, 2017). However, the main mechanisms activated and the morphological changes presented by cells after hormones exposure are poorly understood (Levine, 2017). Endocrine mechanisms underlying wound healing is mainly associated with modulations in TGF-β and α-SMA production (Meng et al., 2016). The activation of these pathways can stimulate ECM production, fibroblast proliferation, cell migration, PDGF secretion and angiogenesis (Marangoni et al., 2015; Monika et al., 2021). Generally, these findings are demonstrated using in vitro analyses, but in vivo studies were also included in this review in search of morphological adaptations in fibroblasts and myofibroblasts. In addition, current evidences are limited in indicating

mechanism of action, cell signaling pathways, doses and time of treatment in which hormones should be used to get the best healing effects. Thus, defining these parameters is a challenge task to be pursued in the search for more efficient regenerative therapies for skin wounds management. Accordingly, this systematic review integrated in vitro and in vivo evidence on the main protocols and effects of hormonal treatments on fibroblasts and myofibroblasts during skin wound repair, delimiting key mechanisms and biomarkers involved in these processes. The methodological quality all studies reviewed was also investigated, pointing out the main sources of bias that compromise evidence quality and that must be overcome in studies involving hormones exposure during skin wound repair.

2. MATERIALS AND METHODS

2.1. Focus question

The main question to be answered in this systematic review were: (i) are there hormone effects on the differentiation of fibroblasts into myofibroblasts during skin repair *in vivo* and *in vitro*? (ii) what are the main mechanisms of action of these hormones in this differentiation process? and (iii) which doses and times of hormone exposition exert the best effects on myofibroblast differentiation?

2.2. Search strategy

This systematic review followed the preferred reporting items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Page et al., 2021). The studies were selected using an advanced search on PubMed/Medline (www.ncbi.nlm.nih.gov/pubmed), Scopus (www.scopus.com), and Web of Science (www.webofknowledge.com) databases, on August 15, 2022 at 12:08 pm. A comprehensive two-phases search strategy was used to retrieve all relevant studies:

i) direct searches in electronic databases, and ii) indirect screening of the reference lists of all studies identified in the direct searches.

For all databases, structured search filters were based on four complementary levels: (i) myofibroblasts, (ii) wound healing, (iii) skin, and (iv) hormones. Search filters were initially developed for PubMed, and the search algorithms [MeSHTerms] and [TIAB] were respectively applied to identify indexed records and those recently published in indexing processes. To detect all in vivo animal studies in PubMed, a standardized and optimized animal filter was obtained. The search terms used in PubMed were adapted for Scopus and Web of Science databases. The “animal model” filter was provided by the Scopus database (Table S1-suplemmentary data). The studies were filtered considering the English, Portuguese, and Spanish languages.

Two reviewers (ADS and MMS) conducted the literature search, removed duplicate records and irrelevant studies based on titles and abstracts screening. After this initial screening, potentially relevant studies were obtained in full text, which were independently assessed for eligibility by two reviewers (ADS and MMS). The selected studies were then compared, and inconsistencies were resolved in consultation with two expert reviewers (RVG and RDN).

Secondary studies (e.g., literature reviews, commentaries, notes, book chapters), unindexed studies, and studies with other target organs (e.g., lung, liver, heart, kidney, bowel, and bone) were excluded. Ex vivo studies, studies that did not investigated fibroblasts, myofibroblasts, hormones not associated with wound healing, and studies evaluating plant-derived hormones were also excluded. Only studies that met the following eligibility criteria were selected: (i) Studies evaluating hormone effects on skin wound healing from in vivo murine models, (ii) Studies evaluating the effect of hormones on murine fibroblasts and myofibroblasts in vitro, (iii) Studies

evaluating fibroblasts and myofibroblasts in wounds samples. An indirect screening of reference lists of all selected studies was performed after selecting eligible studies in electronic databased. The kappa coefficient was calculated using the number of studies independently excluded and included by both researchers (kappa = 0.936).

2.3. Data extraction and management

Publication data were extracted using standardized information such as: **Studies *in vivo***: (i) publication characteristics (e.g., author, publication year, country, ethical approval, statistical analysis) and murine models (e.g., animal strain, sex, age, number of experimental groups, and number of animals per group); (ii) interventions (e.g., hormones, chemical classification, producing gland, administration route, and dose); (iii) wound healing model (incisional or excisional, wounds per animal and evaluation period); (iv) research outcomes (wound area, (myo)fibroblast distribution, cytokines, inflammatory cells, vascularization, extracellular matrix components). **Studies *in vitro***: (i) publication characteristics and cell lines; (ii) intervention (hormones, type of culture, hormone dose, and culture medium), and (iii) research outcomes (e.g., (myo)fibroblast, cell migration, cell contraction, extracellular matrix components and cytokines). Disagreements in data extraction were resolved in consultation with two expert reviewers (RVG and RDN). Data extracted from all studies reviewed are indicated in Table S2.

2.4. Bias analysis

Studies quality was verified using the Risk of Bias (RoB) Review Center for Animal Laboratory Experimentation tool - SYRCLE's (Hooijmans, et al., 2014). In this tool, questions are divided into the following subtopics to facilitate the judgment of scientific articles through the characteristics of animal models: Q1-Q2: selection bias; Q3; performance bias; Q4: detection bias due to knowledge of interventions by

outcome evaluators; Q5: attrition bias (quantity, nature or processing of incomplete results data); Q6: reporting bias due to selective result reporting.

In addition, we added eight questions that contributed to the studies judgment: Q7: conditions under which the animals were kept (e.g., temperature, humidity, light/dark cycles, water and food) were reported?; Q8: allocation information (e.g., individual, collective, how many per allocation) were reported?; Q9: complete information about the intervention (dose, time and interval of exposure of the intervention) were indicated?; Q10: wound closure data were presented with follow-up days, photos and graphics?; Q11: methodology used to obtain the results is reliable, available, and replicable?; Q12: the study reports withdrawals and/or exclusions from any group and the reasons?; Q13: the study was approved by an ethics committee?; Q14: the statistical methods used were reported?; Q15: the study directly addresses the review issue?; Q16: the study has failures or lacks in tables information?.

Articles in the SYRCLE's RoB tool were marked with “yes” (low risk of bias), “no” (high risk of bias), or “unclear” (indicating that the item was not clearly reported and therefore the risk of bias was unknown). The SYRCLE’s chart was built using the Review Manager 5.4 software. A table summarizing all relevant and applicable aspects was constructed considering the specificity and objectives of the systematic review. Individual and general adherence to the bias criteria were expressed as absolute and relative values (Hooijmans et al., 2014).

2.5. Statistical analysis

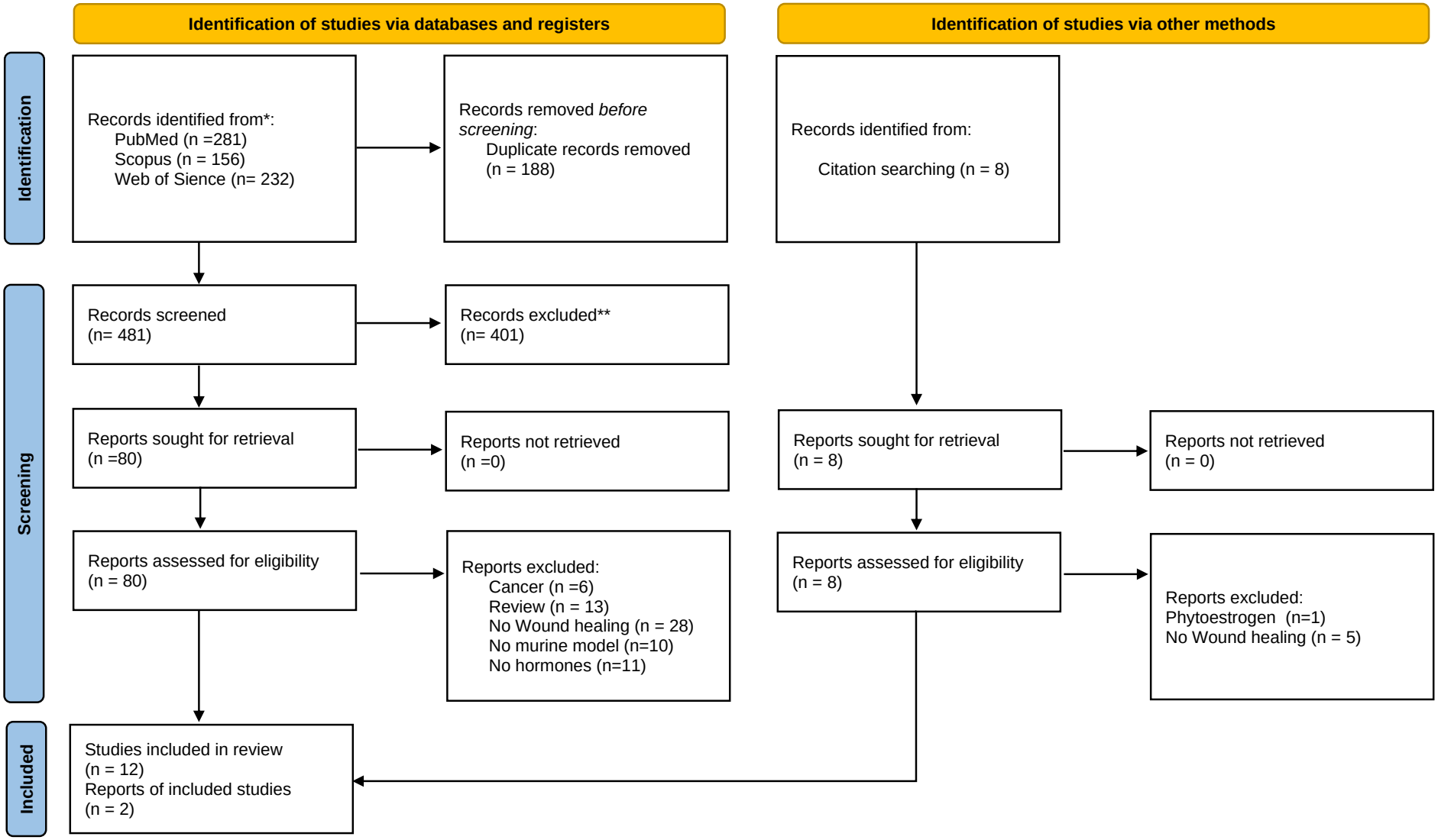
Considering the potential heterogeneity in the identified studies, we used a statistical model based on a weighted mean difference meta-analysis of random effects, in which some heterogeneity and sampling error is allowed to calculate a summary estimate of the effect size and its 95% confidence interval (DerSimonian and

Laird, 2015). For this model, the standard error (SE) was converted to standard deviation using the formula SD = SE × √n; where n is the number of animals used in each experimental group. The variability of each outcome evaluated was presented as a heterogeneity statistic (I2) (Higgins, 2003). For dichotomous (experimental model, treatment time, doses used, and evaluated parameter) and continuous variables, hazard ratio (RR) and standardized mean difference (SMD) were respectively used as effect estimates. Where outcomes were measured repeatedly, we established two wound healing time points (7 and 14 or 3 and 7 day), where the average outcome was calculated for each phase.

3. RESULTS

3.1. Publication characteristics

The initial search resulted in 669 studies, 281 from PubMed, 156 from Scopus, and 232 from Web of Science. Of these, 188 were excluded because they were duplicate. After reading the titles and abstracts, 80 relevant studies were selected and read in full-text, and their references were checked. Thus, 12 studies fully met the inclusion criteria and were included. After reading the reference list of all selected articles, 2 relevant articles were found, totaling 14 articles included in this review. The article selection process is shown in a figure 1 (PRISMA flowchart).



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Of the 14 selected studies, 12 (85.7%) used only *in vivo* models, and 2 (14.2%) used *in vivo* and *in vitro* models. These studies were carried out in nine different countries: Japan, United Kingdom, and Brazil (21.4%, n=3 each), Germany (14.2%, n=2) and the England, Greece, and Slovak Republic (7.1%, n=1 each) (Table S2).

3.2. Preclinical studies with animal models

3.2.1. Animal Model Characteristics

From all *in vivo* studies, C57BL/6 mice (50%, n=7), Sprague-Dawley rats (28.6%, n =4), Swiss mice (14.2% n=2), and Wistar rats (7.1% n=1 each) were used as experimental animal models. Of these, wild type animals (64.3%, n=9) and transgenic/knockout (35.7%, n=5) were used. Six studies used males (42.8%), 6 studies used females (42.8%) and 2 studies (14.2%) used males and female animals in the same experiment. Animals age ranged from 6 to 12 weeks in 9 studies (64.2%), 1 study (7.1%) reported adult age and 5 studies (35.7%) did not report this information. Animals weight was not specified in most studies (85.7%, n=12). Only 1 study (7.1%) with mice presented this information (25-30g) and 1 study (7.1%) reported rats weight ranging from 200 to 259 g. All studies (100%) reported animals' number per experimental group (Table S2).

3.2.2. Wound characteristics

Nine studies (64.2%) evaluated excisional wounds, 4 studies (28.5%) excisional and incisional wounds, and only 1 study (7.1%) incisional wounds. All the wounds (100%) were performed on the animal's back. All studies (100%) reported the wound number, which ranged from 1 to 6 per animal. The wounds area ranged from 4mm to 10mm diameter. The interval for tissue removal from wounds was reported in all studies (100%) ranging from 0 to 21 days. Ketamine and Xylazine (42.8%, n=6) were the most used anesthetics, followed by isoflurane and pentobarbital (21.4%, n=3 each),

and 1 study (7.1%) used tiletamine chloride. Hormonal exposure characteristics was not report in 1 study (7.1%) (Table S3).

3.2.3. Treatment characteristics from in vivo studies

To assess hormonal effect on myofibroblasts distribution, 8 in vivo studies used gonadectomized animals (57,1%), 4 performed hormonal blockade (28.5%), 1 study (7.1%) used hormonal overexpression and, 1 study (7.1%) used hormonal agonists. Only 3 studies (21,4%) administered hormones using topical or oral applications, and 7.1% (n=1 each) administered both subcutaneously and intraperitoneally. Considering all studies (n=14 or 100%) adopting hormonal blockade, overexpression, or castration, only 5 studies (35,7%) confirmed hormone levels, 4 studies (28,5%) did not reported this information, and 2 studies (14,2 %) reported that hormones were quantified but did not present these results.

From all studies evaluated, 11 (78.6%) used lipid hormones, and 3 (21.4%) used peptide hormones. Testosterone (7.1%, n=1) and dihydrotestosterone (DHT) (21.4% n=3), estrogen and testosterone (7.1%, n=1); propyl (1H) pyrazole-1,3,5-triyl-trisphenol (PPT- ER-α agonist) (7.1%, n=1); 17-β estradiol (28.6%, n=4), dexamethasone (cortisol agonist) and glucocorticoids (7.1%, n=1 each) were the lipid hormones investigated. Growth hormone-releasing hormone (GHRH), glucagon-like peptide-1 (GLP-1), and growth hormone (GH) (7,1%, n=1 each) were the peptide molecules investigated (Table S4).

3.2.4. Main in vivo outcomes

From studies evaluating lipid hormones, it was observed that 1mg/kg PPT (ER-α agonist) increased α-SMA labeling, indicating that this hormone exerted a positive role in myofibroblasts differentiation. Conversely, 0.05 mg/kg 17-β estradiol did not

influence myofibroblasts labeling. However, 10 mg/kg 17-β estradiol and 2 mg/kg dexamethasone increased myofibroblasts immunostaining (α-SMA labeling) compared to the control animals at 14 days healing. This effect was evaluated from testosterone, DHT, and glucocorticoids, blockade, or overexpression. The effect of lipid hormones on wound healing was most commonly assessed over 7-14 days, and only one study assessed 21 days.

Considering peptide hormones with 3 mg/kg GLP-1 activator increased myofibroblasts marking for in the wounds, and the same result was found after 10 to 13 days 100 nM GHRH exposure (Table 1). In addition, 500nM of GHRH was administered *in vitro*, the immunostaining of myofibroblasts was reduced, indicating a dose-dependent effect. Peptide hormones effect on wound healing was investigated during 10 to 14 days. The GH hormone was evaluated for overexpression and inhibited immunostaining for myofibroblasts (Table 1).

Table 1: Main results reported in *in vivo* studies included in the systematic review on endocrine stimulation in the process of differentiation of fibroblasts into myofibroblasts in wound healing.

Reference	Hormone	Dose	Main results	
Almeida Tais et al., 2016	Glucocorticoids (GCs)	20 mg/kg	Myo (=) WA (+)	Mϕ (=)
Campbell et al., 2010	17-beta estradiol (17β-E)	0.05 mg/kg	Myo (=) WA (-)	
Dioufa et al., 2010	Growth hormone-releasing hormone (GHRH)	100 nM	Myo (+) WA (-)	Col (+) Inf (+)
Marchioni et al., 2010	Dexamethasone (Dx)	2 mg/kg	Myo (+) WA (?)	PMN (-)
Mukai et al., 2014(A)	17-beta estradiol (17β-E)	10 mg/kg	Myo (+) WA (-) Col (+)	TNF-α (=) Mϕ (-) M2(+)
Mukai et al., 2014(B)	17-beta estradiol (17β-E)	10 mg/ kg	Myo (+) WA (-)	Col (+) TNF-α (=)
Mukai et al, 2014(C)	17-beta estradiol (17β-E)	10 mg/kg	Myo (+) WA (-)	Col (-)
Romana-Souza et al., 2013	Testosterone (Tes) and Estrogen (E)	-	Males (CSX) Myo (-) WA (-) Hyd (=) Ang (+) Rep (+) VEGF (+) Mϕ (-)	Females (OVX) Myo (=) WA (=) Hyd (-) Ang (+) Rep (=) VEGF (=) Mϕ (+)

Schurmann et al., 2011	Glucagon-like peptide (GLP-1)	3 mg/kg	Myo (+) WA (-)	Ang (=) PMN (-)
Gilliver et al., 2005(A)	Dihydrotestosterone (DHT)	-	Myo (=) WA (-) IL-6 (-)	TNF-α (-) TGF-β (+)
Gilliver et al., 2007(B)	Dihydrotestosterone (DHT)	-	Myo (=) WA (+)	Col and FIB (+) MMP 2 e 9 (-)

(+) Increase; (-) Decrease; (=) There Was no difference; IP: Intraperitoneal; Sub: subcutaneous; Top: Topic; Ang: Angiogenesis; Col: Colagen ; CSX: Neutered; FIB: Fibronectin; IL: interleucin; Hyd: Hidroxiprolin; Infl: Inflammatory infiltration; IGF1and IGFBP: Insulinoid growth factors; Myo: Myofibroblast; MMP: Metalloproteases; Mϕ: Macrofage; M2 : Macrofage 2; OVX: Ovariectomized; PMN: Polymorphonuclear; Rep: re-epitalization; TGF-β: Transforming Growth Factor-β; TNF-α: Tumor neurosis factor; VEGF: Vascular endothelial growth factor; WA: Wound area.

3.3. Preclinical studies using in vitro models

3.3.1. Characteristics of cell culture

As reported in Table S5, 2 *in vitro* studies evaluating hormonal interference on myofibroblast differentiation were identified. Dermal (50%, n=1), carotid and pulmonary (50%, n=1 each) fibroblasts were used. One study (50%) used primary culture and 1 study (50%) used immortalized cells. All studies were performed with murine strains. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (50%, n=1), and 1 study cultured the fibroblasts in stressed collagen lattices (50%, n=1).

3.3.2. Characteristics of in vitro hormonal exposure

In vitro studies study evaluated GH or GHRH (50%, n=1). All cell types used were murine, including pulmonary (50%, n=1) and dermal (50%, n=1) cells. GHRH has evaluated at 100 nM and 500 nM for 1 day. GHRH increased α-SMA expression by up to 3× at 100 nM compared to control cells. However, this marker was reduced at 500 nM. α-SMA immunostaining was reduced after 12.3, 61.3 and 245 ng/mL GH administration for 5 days (Table S5).

3.4. Main preclinical evidence

3.4.1. In vivo studies and lipid hormones

From *in vivo* studies, 5α-reductase inhibitor prevented enzyme activity in converting testosterone to DHT (the active testosterone form myofibroblasts immunostaining (anti-α-SMA) was unaffected by this inhibitor compared to untreated control animals. However, myofibroblast density (α-SMA labeling) was reduced in castrated animals (low testosterone levels) compared to intact (non-castrated) animals with normal testosterone levels. Conversely, collagen I levels (a ECM component) were increased, while MMP-2 and MMP 9 activities (collagenolytic enzymes) were reduced in the castrated animals, suggesting that testosterone attenuates ECM

degradation. Blood vessels distribution in the scar tissue was also increased by testosterone in intact males (microscopic analysis). Estrogen and 17-β estradiol also increased α-SMA labeling, indicating greater efficacy in stimulating fibroblast differentiation.

Considering a quantitative analysis, studies with methodological similarity (e.g., experimental model, treatment time, hormone doses and research outcomes) were grouped. Studies with high similarity were pooled and selected for meta-analysis. From this method, we identified that 10 mg/kg 17-β estradiol did not influence α-SMA labeling in the scar tissue collected 7 days after skin injury (mean value 1.04, confidence interval [-0.36, 2.44], P=0.07). However, this treatment increased α-SMA immunostaining in the scar tissue collected 14 days after skin injury (mean value -4.29, confidence interval [-7.01, -1.58], P=0.04) (Figure 2).

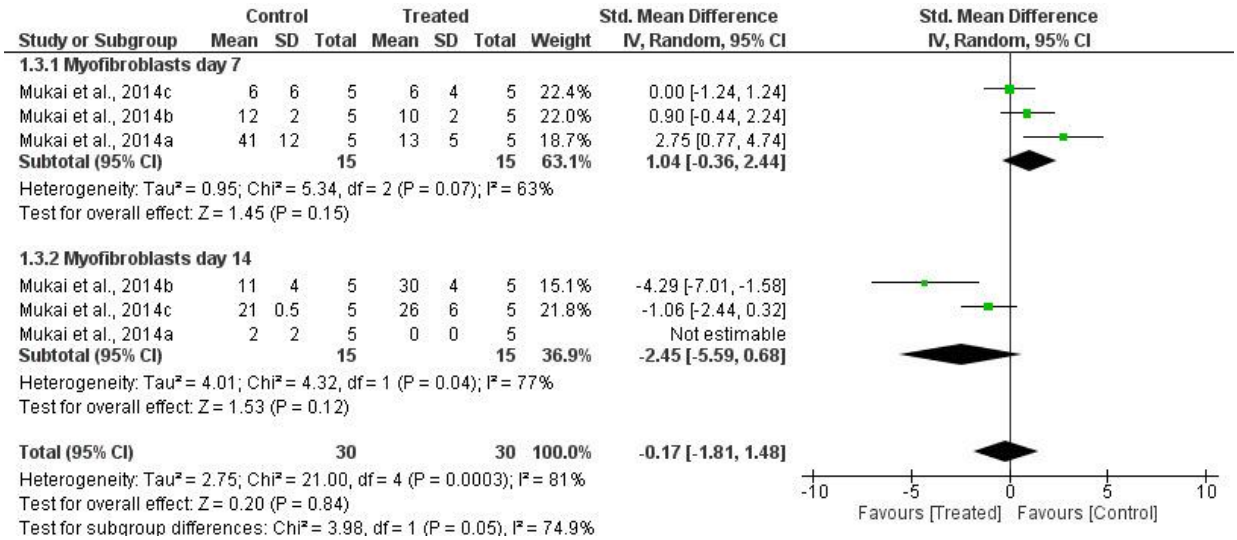


Figure 2: Forest plot obtained from a meta-analysis evaluating the influence of 17-b estradiol on the immunostaining of myofibroblasts in the healing of skin wounds in 7 and 14 days. Comparisons were made between treated and untreated groups (control).

Estrogen and 17-β estradiol also increased total collagen and hydroxyproline levels in the scar tissue. Furthermore, the results of the meta-analysis indicated that 17-β estradiol stimulated collagen deposition in the early (7 days) and late (14 days)

phases of wound healing (mean value 10.25, confidence interval [3.95, 16.55], P=0.0001) (Figure 3). Reduced wound area and increased tissue vascularization during the proliferative phase were also observed in animals receiving estrogen and 17-β estradiol (Table 1).

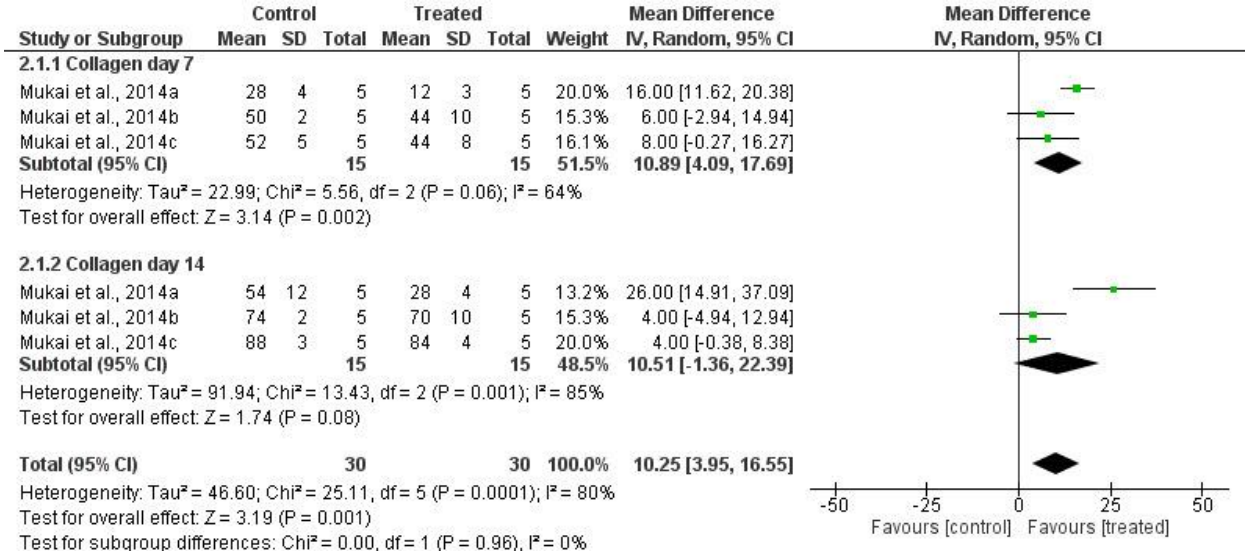


Figure 3: Forest plot obtained from a meta-analysis evaluating the influence of 17-b estradiol in stimulating total collagen deposition in cutaneous wound healing in 7 and 14 days. Comparisons were made between the treated and untreated groups (control).

In addition, cells and cytokines were we also analyzed. Accordingly, 17-β estradiol increased M2 macrophage labeling (CD163 positive) in the scar tissue 7 days after skin injury, which are cells directly involved with anti-inflammatory responses. Conversely, neutrophil recruitment was reduced by this treatment. Despite these results, our meta-analysis results did not indicate an general effect of 17-β estradiol on macrophages (mean value -336.63, confidence interval [-808.62, 135.37], P=0.0002) and neutrophils (value of mean -310.76, confidence interval [-765.42, 143.89] P=0.00001) recruitment. This result can be partially explained by I^2 value (Macrophages - 3 days= 84%, 7 days= 88%; Neutrophil - 3 days= 87%, 7 days= 91%), which indicated high variability between the studies compared. Apparently, treatment

times and animal models used were the main sources of methodological variability identified, which had a potential impact on meta-analysis results (Figure 4).

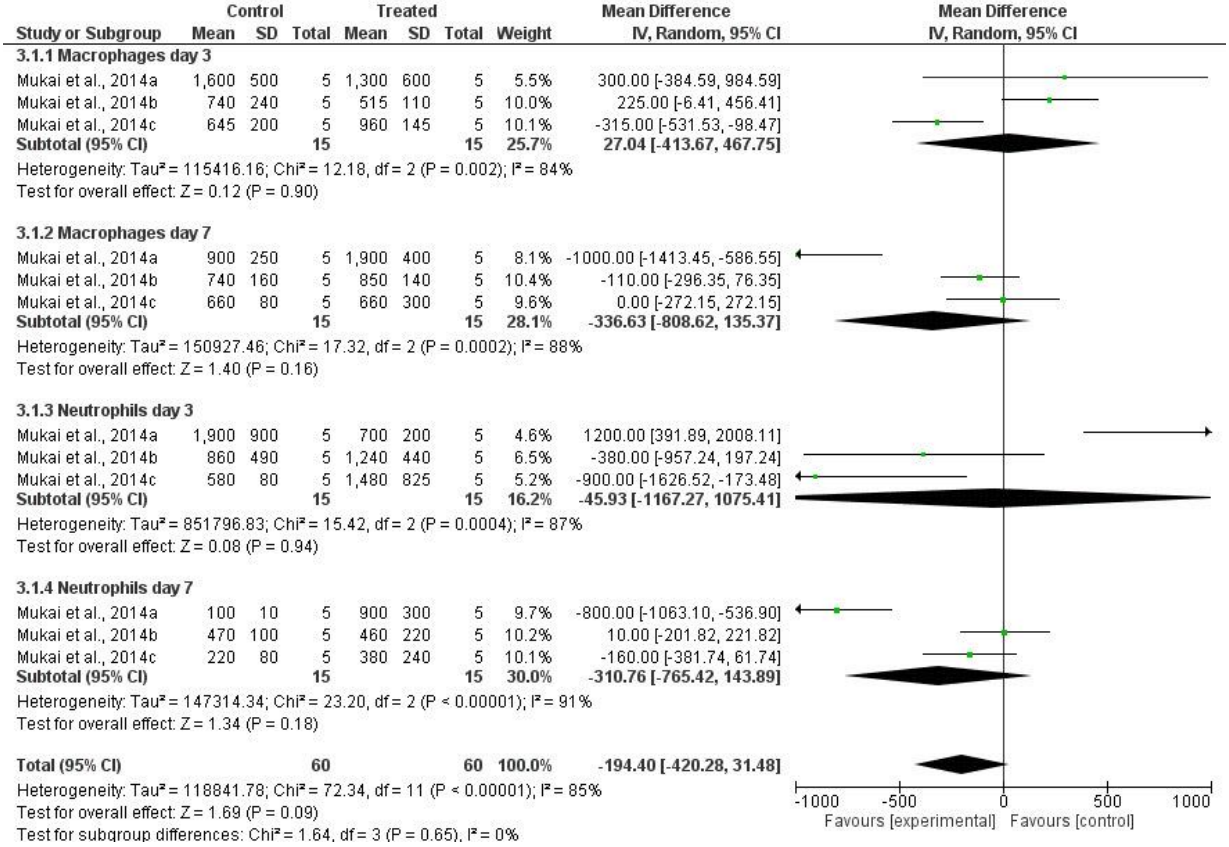


Figure 4: Forest plot obtained from a meta-analysis evaluating the influence of 17-b estradiol in stimulating the recruitment of macrophages and neutrophils in the healing of skin wounds in 3 and 7 days. Comparisons were made between the treated and untreated groups (control).

Furthermore, TNF-α levels were unaffected by 17-β estradiol (Table 1), as reinforced by meta-analysis (I^2 value: 3 days= 18%, 7 days= 98%) (Figure 5). However, TNF-α and IL-6 immunopositive cells were increased by testosterone administration (Table 1).

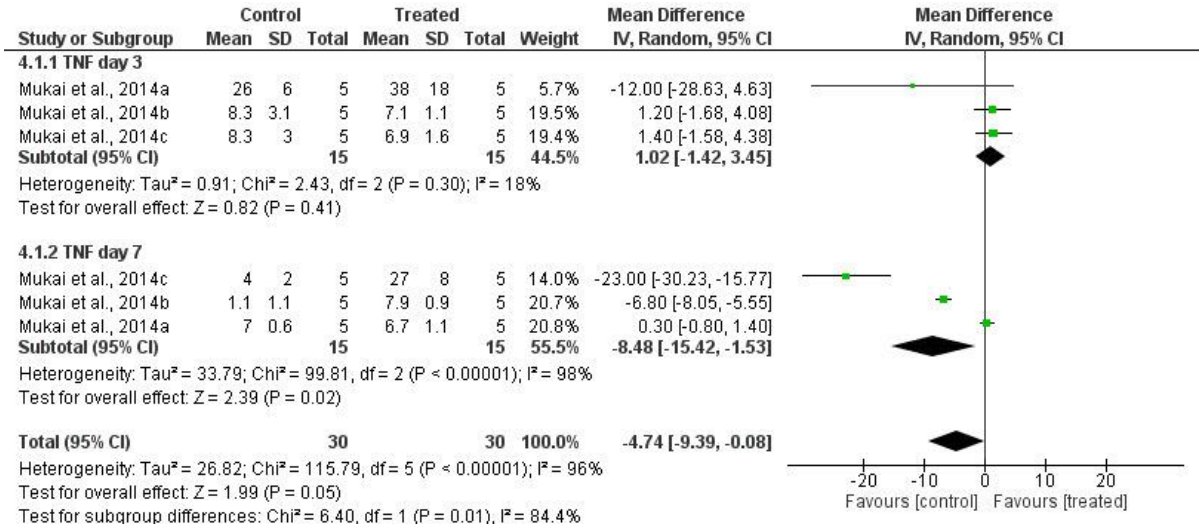


Figure 5: Forest plot obtained from a meta-analysis evaluating the influence of 17-b estradiol in stimulating TNF-α synthesis in the healing of skin wounds in 3 and 7 days. Comparisons were made between the treated and untreated groups (control).

3.4.2. Tissue and cellular mechanisms involved on the myofibroblasts differentiation after hormone exposure

3.4.2.1. In vivo studies and lipide hormones

Dihydrotestosterone and testosterone stimulated different events during tissue repair. Testosterone stimulated myofibroblasts differentiation by activating mesenchymal endothelium transition (EndoMT). However, DHT blockeage exerted no impact on this process or contractile responses. Wound healing was accelerated from 5-*α*-reductase (DHT activator) inhibition. This effect occurred because DHT binds to the androgen receptor (AR) and is activated by Smad3, increasing pro-inflammatory cytokines production by macrophages at the same time that attenuates IL-6, TNF-α and TGF-β expression by fibroblasts. Thus, TGF-β justifies reduced myofibroblasts differentiation promoted by DHT (Figure 6).

On the other hand, estrogen, and 17β-estradiol reinforced myofibroblasts distribution via estrogen receptor alpha (ER-α) stimulation. ER-α is an intracellular estrogen receptor, which is phosphorylated and translocated to the nucleus after

hormonal stimulation. Estrogen binds to ERα, which binds to an adapter protein (collagen homolog Src (Shc)) forming a protein complex (ERα-Shc). This complex activates MAPK pathway, which activates genes transcription such as SM22-α (α-SMA) and COL1A1 (collagen type I alpha I), modulating collagen production in addition to activating genes responsible for cell proliferation and migration while inhibiting MMP expression.

Glucocorticoid hormones demonstrated its positive effects on myofibroblasts considering that dexamethasone stimulated α-SMA expression via MAPK activation. However, dexamethasone reduced collagen production and fibroblasts distribution of cytoplasmic organelles, such as Golgi complex and rough endoplasmic reticulum, demonstrating a negative effect of this drug on matrix remodeling and tensile strength (Figure 6).

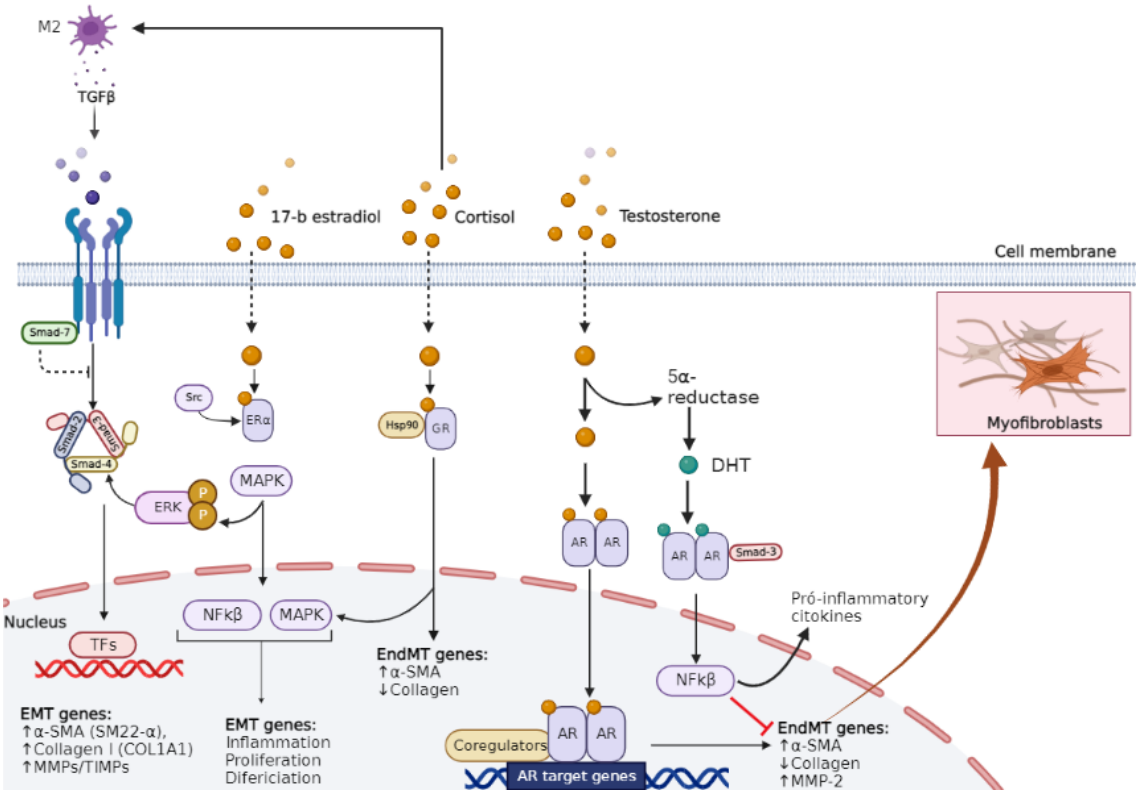


Figure 6: Schematic diagram showing the main mechanisms involved in the stimulation of lipid hormones in the differentiation of fibroblasts into myofibroblasts in wound healing.

3.4.3. In vivo studies and peptide hormones

In studies using peptide hormones, GH overexpression reduced α-SMA labeling in 5 days, showing that this hormone overexpression negatively modulates the conversion of fibroblasts into myofibroblasts. However, the authors reported an increase IGF-1 (a co-stimulator of cell differentiation). GH overexpression elevates IGF-1 levels, which acts as negative feedback on the somatotropic axis, inhibiting GHRH and consequently this imbalance reduces GH release. In addition, GH overexpression promoted wider wounds filled with excessively dense and thick granulation tissue, decreasing tissue strength. On the other hand, the action of (GHRH), when applied topically to the wound, promoted immunostaining for myofibroblasts. GHRH binds to its SV1 membrane receptor, stimulating TGF-β expression and activating Smad pathway-dependent α-SMA transcription (Figure 7).

The enzyme DPP-4, expressed on the cell surface, inhibits hormone glucagon-like peptide-1 (GLP-1) effects. DPP-4 blockage stimulates GLP-1 action, which upregulates α-SMA gene expression. In addition, COX-2 gene expression is reduced by GLP-1. COX-2 activates pro-inflammatory pathways and is associated with M1 macrophages polarization, which produce toxic intermediates such as reactive species (ER) and nitric oxide (NO). The degradation of cell membrane phospholipids by ER generates arachidonic acid, which together with COX-2 originate prostaglandins, which activate pro-inflammatory pathways. Furthermore, macrophage inflammatory protein-2 (MIP-2) labeling indicates that GLP-1 stimulates M2 mcrophages differentiation. M2

macrophages are alternatively activated cells, which produce anti-inflammatory cytokines, stimulating EM production and wounds contraction, (Figure 7).

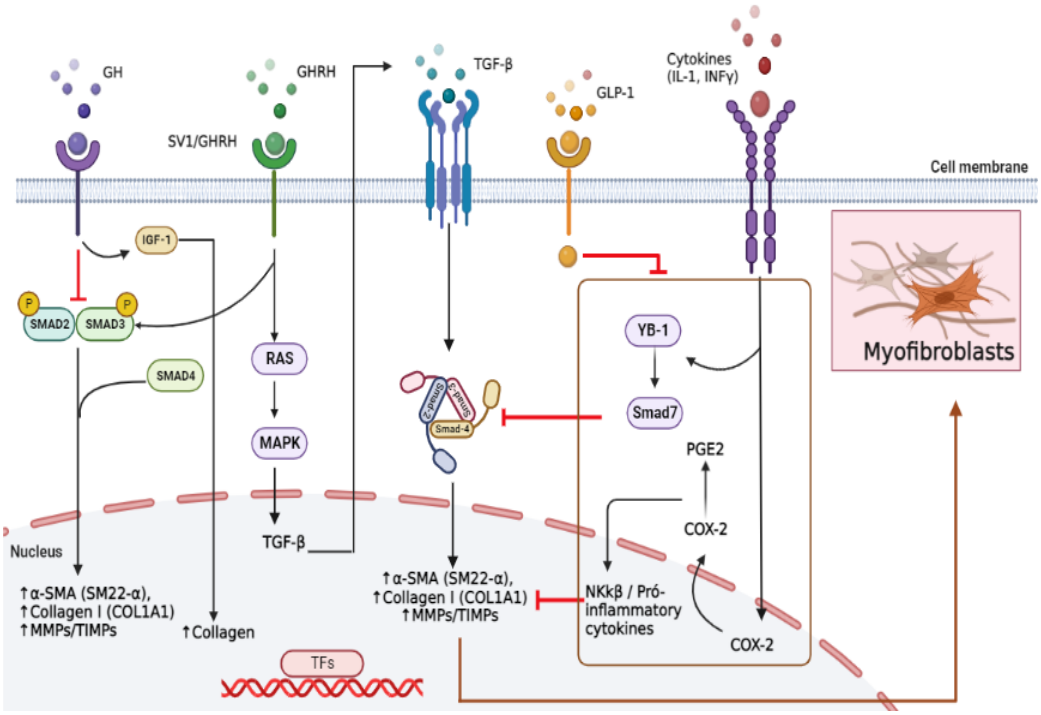


Figure 7: Schematic diagram showing the main mechanisms involved in the stimulation of peptide hormones in the differentiation of fibroblasts into myofibroblasts in wound healing.

3.4.4. In vitro studies

Corroborating the *in vivo* results, GHRH also increased α-SMA expression in addition to increasing cell migration via TGF-β activation. On the other hand, α-SMA immunostaining was reduced by GH treatment. However, this hormone exerted no inhibitory effect on collagen biosynthesis, indicating the contraction potential in a dose-dependent manner (0.1 to 10%) (Figure 8).

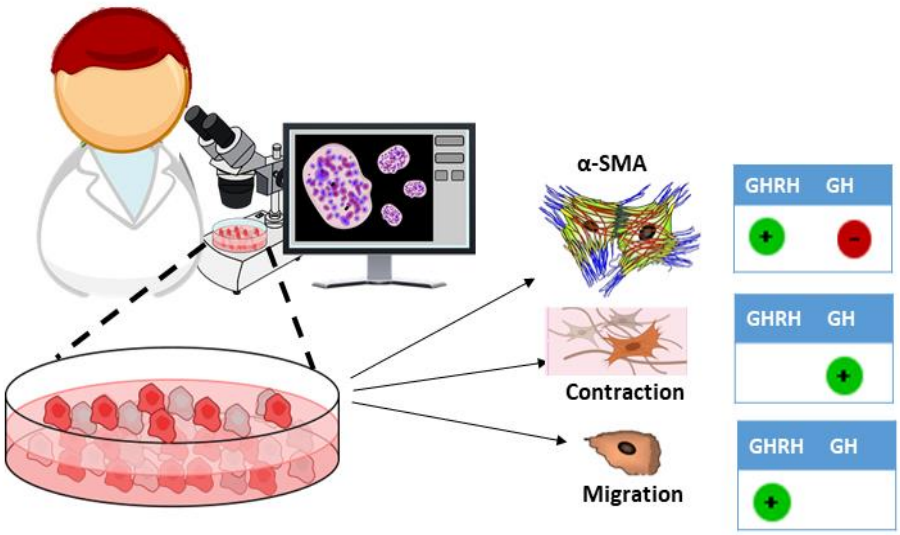


Figure 8: Schematic diagram showing the main results of endocrine stimulation in the differentiation of fibroblasts into myofibroblasts *in vitro*.

3.5. Methodological quality assessment

The detailed results of the bias analysis are shown in Figures 9A and B. No study met all methodological criteria analyzed. Regarding selection bias, the sequence generation process was not reported in 50% of the studies (n=7), and allocation concealment was also not reported in most studies 57.1% (n=8). Considering performance and detection bias, blinding of outcome raters was overlooked in 64.2% (n=9) studies. Regarding attrition bias and reporting bias, 14.2% of all studies presented incomplete or selective results (n=4 each). Three studies (21.4%) did not report animal characteristics, such as biometric information and age. Six studies (42.8%) did not inform whether the allocation of animals was individual or collective. The treatment protocol was reported in all studies. Only 7.1% (n=1) of the studies did not report wound closure data. Adequate analytical tools were reported in 11 studies (78.5%), but this information was unclear in 3 studies (21.4%). Removal of animals or deletion of data was not reported in all studies. Ethics committee approval was not reported in 3 studies (21.4%). The statistical method was not reported in 1 study

(7.1%), while most studies (92.8%, n=13) used adequate statistical tests. Unclear results were identified in 1 study (7.1%).

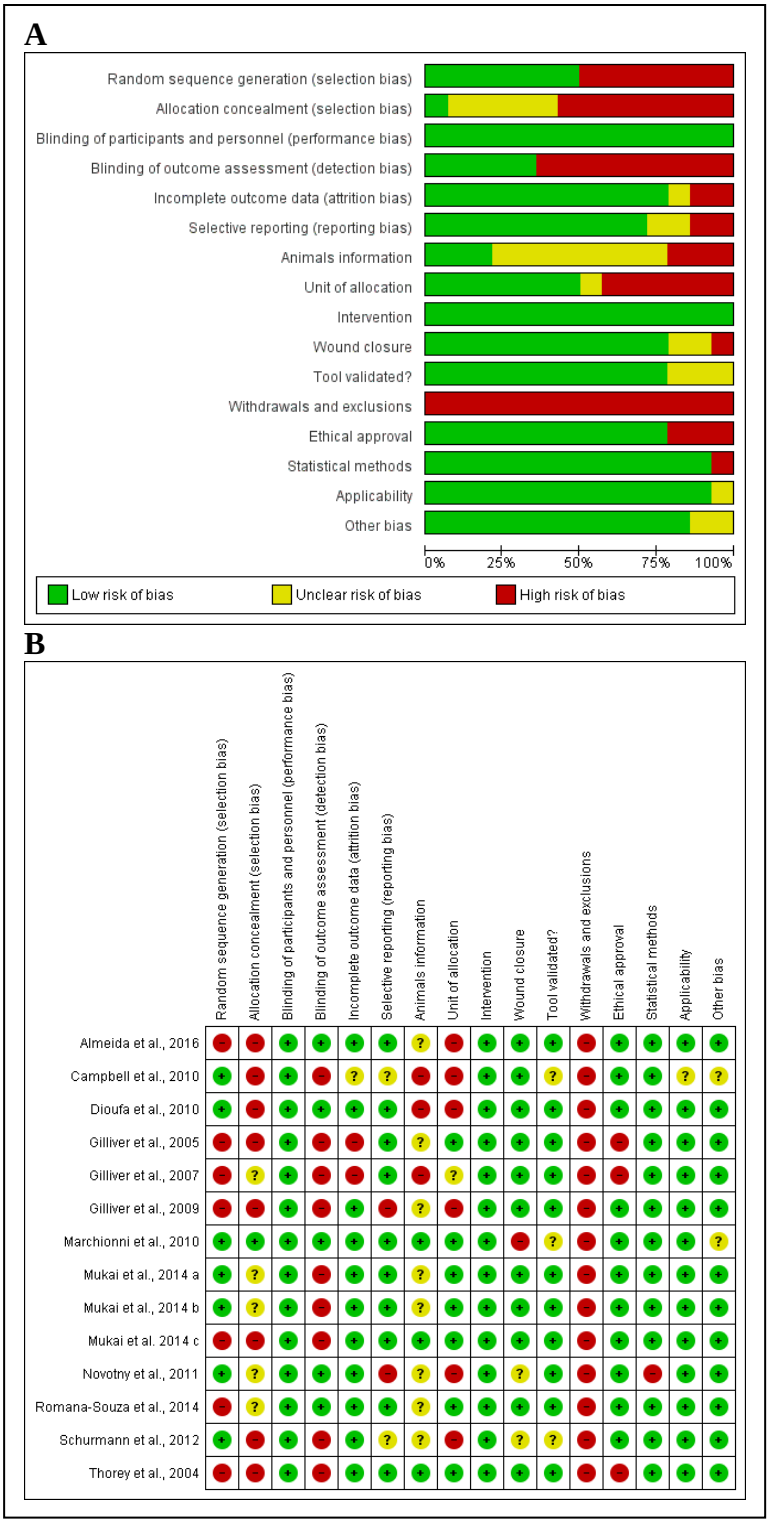


Figure 9: Results of risk of bias and methodological quality indicators at the individual level (A) and all studies included in this systematic review (B) that evaluated endocrine stimulation in regulating the differentiation of fibroblasts into myofibroblasts in cutaneous wound healing. The items in the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias assessment were scored with “yes” indicating low risk of bias, “no” indicating high risk of bias, or “unclear” indicating that the item was not reported, resulting in an unknown risk of bias.

4. DISCUSSÃO

The use of hormones in regenerative medicine, especially for tissue repair, has been widely investigated (Caicedo and Devesa, 2019; Chainy and Sahoo, 2020; Schally et al., 2019). However, several hormonal effects and mechanisms of action are still unclear, mainly due to complex interactions between target organs and their receptors (Bao et al., 2014; Nicholson and Johnston, 2005). Understanding molecular and cellular interactions of hormones during wound healing is of paramount importance to clarify specific therapeutic effects, which can improve tissue repair and reduce treatment time. Thus, we developed a systematic review to investigate how lipid and protein hormones can influence fibroblast differentiation into myofibroblasts and the main mechanisms activated during this process using *in vitro* and *in vivo* models. Our results indicated that hormones-induced fibroblast differentiation is related with TGF-β and anti-inflammatory cytokines released, especially by macrophages. These mechanisms are potentially blocked by DHT, impairing fibroblast differentiation into myofibroblasts. However, testosterone, estrogen, 17-b estradiol, and dexamethasone stimulated this process. Conversely, the peptide hormone GH prolonged the inflammatory phase, inhibiting fibroblasts differentiation into myofibroblasts by

modulating TGF-β expression. In addition, inflammatory markers were down-regulated and α-SMA expression was increased by GLP-1 and GHRH, which improved wounds contraction.

Our studies date back to 2001 for *in vivo* studies and 1998 for *in vitro* assays. It's important to highlight that *in vitro* and *in vivo* studies are used at different stages of research development with different complexity degrees. *In vitro* studies are used as filters, selecting the best products to be tested *in vivo*, which also works as a filter to provide tips on efficacy and safety before clinical trials (Peneda Pacheco et al., 2021). From *in vivo* studies, C57BL6 mice were mainly used as animal model. Mice are relevant preclinical models as they are economical, easy to handle, and amenable to genetic manipulation, which favors comprehensive molecular and mechanistic investigations (Shrivastav et al., 2018). Regarding wounds characteristics, most studies only used excisional wounds or combined excisional and incisional wounds on the animals back. In fact, excisional and incisional wounds in the animals back are the most popular models used to investigate the natural healing processes. These models prevent the animal from reaching and manipulating the wound, and allow evaluating the time-dependent healing process from multiple wounds and treatment and control can be performed in the same mouse (Grada et al., 2018).

4.1. Characteristics of hormonal exposure from in vivo and in vitro studies

To assess hormones interference on myofibroblasts development during wound healing, most *in vivo* studies performed gonadectomy (castration or ovariectomy). Murine gonadectomy is used to elucidate biological mechanisms activated after hormones exposure. This method is also relevant to investigate several comorbidities potentially concurrent with skin wound healing, such as bone alterations (Sinha et al., 2016), reproductive and non-reproductive disorders (Chuffa et al., 2017; Gonsioroski

et al., 2020). However, hormone levels were underreported or not investigated in 50% of the studies reviewed, a important methodological limitation.

Most *in vivo* and *in vitro* studies reviewed investigated lipid hormones, particularly testosterone, DHT, 17β-estradiol, estrogen, and corticosteroids. However, investigating the hormonal effects on fibroblasts was a hard task, especially considering that hormonal doses and treatment time were highly variable in most studies, also considering that dose-response was not always investigated. However, studies investigating topical or oral administration showed that low hormone doses such as 0.05 mg/kg do not stimulate fibroblasts differentiation into myofibroblasts, delaying wound closure for 21 days follow-up. Conversely, doses ranging from 1 to 10 mg/kg stimulated myofibroblasts differentiation and a fast wound closure within 14 days. In addition to sexual functions (Aydın and Winters, 2016; Zhu et al., 2019), steroid hormones play an important role in cell growth and differentiation, directly interfering with wound healing (Caicedo and Devesa, 2019). However, knowledge about dosages and therapeutic monitoring of steroid hormones has become basic requirements to assess the efficacy and possible side effects of new hormonal technologies (Conklin and Knezevic, 2020).

Considering peptide hormones, a positive modulation in myofibroblasts differentiation was observed at low doses. Thus, 3 mg/kg a dipeptidyl peptidase-4 (DPP-4) blocker increased GLP-1 hormone availability and improved myofibroblasts immunostaining 10 days after treatment. In addition, topical administration of 100 nM GHRH also stimulated myofibroblasts immunostaining 13 days after hormonal stimulation. Peptide hormones have been increasingly used in different therapies as they are higly selective, used in low dosages, and rapidly eliminated; reducing the risk of off-target effects and systemic toxicity (Muttenthaler et al., 2021).

4.2. *Main preclinical evidence*

Testosterone can be converted in many tissues to its two active metabolites, 5α-dihydrotestosterone (DHT) via 5α-reductase and estradiol by the aromatase enzyme, a product of the CYP19A1 gene (Eriksson et al., 2018). Thus, testosterone conversion to DHT and estradiol may vary mainly due to polymorphisms of genes encoding 5α-reductase and aromatase enzymes (Li et al., 2010). In this study, the evidence indicated that testosterone stimulates and DHT attenuates α-SMA expression, indicating divergent effects of these hormones on myofibroblasts differentiation, TGF-β and pro-inflammatory cytokines expression by fibroblasts. It is already known that myofibroblasts can originate from different cell types (Desmouliere et al., 2014; Marangoni et al., 2015). Our results showed that TGF-β levels, the main myofibroblast differentiation effector, did not differ between uncastrated and castrated animals, while blood vessels distribution was increased in uncastrated males. These findings suggested that the higher myofibroblasts immunostaining in uncastrated animals is potentially related to the complex process of endothelial to mesenchymal transition (EndoMT), in which endothelial cells can differentiate into myofibroblasts (van Caam et al., 2018). Despite having stimulated myofibroblasts differentiation, testosterone reduced collagen I levels and increased MMP-2 and MMP-9 activity, indicating a realistic potential to degrade ECM components and delay wounds closure. Both collagen and MMPs are produced by myofibroblasts and are important in the wound healing (Desmouliere et al., 2014; Hinz, 2016). MMPs degrade collagen and therefore contribute to tissue remodeling. Their activity can be regulated at the transcriptional level or by inhibition by tissue inhibitors of MMPs (TIMPs) (Chow et al., 2012). However, increased MMPs production and activity can induce a marked

imbalance in collagen synthesis and degradation, resulting in excessive collagenolisis and a fragile scar tissue with reduced tensile strength.

Considering similarities in the experimental models, treatment time, hormonal doses, and research outcomes; meta-analysis estimates were calculated to evaluate the impact of endocrine effects on myofibroblasts differentiation. All common and relevant endpoints, such as hormone administered (17-β estradiol), dose, myofibroblasts labeling, total collagen and hydroxyproline content, and inflammatory markers such as M2 macrophages, neutrophils, and TNF-α, reported in three or more studies were combined and quantitatively analyzed. Taken together, meta-analysis results indicated that 10 mg/kg 17-β estradiol stimulated myofibroblast differentiation, collagen formation, and hydroxyproline deposition. Steroid hormones are complex lipophilic molecules, which regulate different cellular functions and move freely into cells, where they activate nuclear receptors that function as transcriptional regulators (Cole et al., 2019). The estrogen signaling pathway can be activated by estrogen binding to its intracellular receptor (ER-α) (Dimauro et al., 2021), which binding to the adapter protein Src and translocates to the nucleus, interacting with target genes that control inflammation, cell proliferation, survival and differentiation (Cole et al., 2019; Dimauro et al., 2021; Meijles et al., 2020). This binding induces the rapid NFκB and MAPK activation, regulating estrogen target genes expression (Dimauro et al., 2021). Furthermore, ERα/Src protein complex activates MAPK, which can phosphorylate ERα at the serine118 site to initiate MAPK-related genes transcription (Yu et al., 2012).

It is already known that MAPK can activate ERK and the ERK/MAPK signaling pathway (Guo et al., 2020), which phosphorylates Smad2/3/4 stimulating the transcription of genes involved in myofibroblast differentiation, cell proliferation and ECM biosynthesis (Chapnick et al., 2011; Meng et al., 2016; Weng et al., 2020).

Another pathway of Smad2/3/4 phosphorylation occurs in response to TGF-β (Ard et al., 2019), being mediated by the type II/I TGF-β receptor. Thus, after Smad2/3/4 phosphorylation, this complex translocates to the nucleus to induce SM22-α gene transcription (gene that characterizes myofibroblasts) (Ard et al., 2019; Lin et al., 2012). In addition, the Smad2/3/4 complex stimulates genes such as COL1A1 for collagen I formation (Kim et al., 2014; Meijles et al., 2020), thereby inducing myofibroblast activation and ECM deposition (Meng et al., 2016). So, after to analyze the individual studies included in our systematic review, it is possible to conclude that MAPK and NFKb pathways should be evaluated to understand lipid hormones influence on skin wound healing.

Cortisol was a lipid hormone additionally investigated. The glucocorticoid cortisol, as well as dexamethasone (a synthetic glucocorticoid), have an important anti-inflammatory role, facilitating the production of anti-inflammatory cytokines such as TGF-β and IL-10 by M2 macrophages (Fernandes et al., 2020; Ingawale and Mandlik, 2020). In our review, these hormones increased α-SMA levels, suggesting that this effect is potentially related with M2 macrophages participation in the inflammation resolution phase, secreting TGF-β, the main myofibroblast differentiation inducer. In addition, glucocorticoids bind to intracellular glucocorticoid receptors (GR), which are bound to stabilizing proteins including heat shock protein 90 (Hsp90), without which the receptor cannot bind the hormone and is consequently unable to affect gene transcription (Sevilla and Pérez, 2018). Thus, cortisol is known for its anti-inflammatory action, regulated by NFKβ and MAPK pathways (Dong et al., 2018). However, dexamethasone treatment reduced organelles development and collagen synthesis by fibroblasts, indicating that this hormone can impair tissue remodeling phase, especially because collagen synthesis is essential in the healing process. Thus,

collagenogenesis inhibition can delay tissue repair and reduce scar tissue tensile strength (Gonzalez et al., 2016; Pakshir et al., 2020).

In addition, *in vivo* and *in vitro* models indicated that GH myofibroblasts labeling, while GHRH increased this marker. It is already known that GH production by the pituitary gland is mainly regulated by the counter-regulatory effects of the hypothalamic hormones GHRH (Schally et al., 2019). However, our findings showed that GH overexpression increased IGF-1 levels, which promoted a negative feedback regulation of GH production by GHRH (Al-Samerria and Radovick, 2021). IGF-1 stimulates the synthesis of collagen, glycosaminoglycans and proteoglycans by dermal fibroblasts (Talebpour Amiri et al., 2014), which justifies the increase in granulation tissue presented by the skin wounds of animals that overexpress GH. However, Saranac et al., (2016) reported that IGF-1 acts as a blocker of the TGF-β/Smad pathway by inhibiting the differentiation of fibroblasts into myofibroblasts (Sarenac et al., 2016). Chow et all reported that the hormone relaxin, a peptide hormone, inhibits the differentiation of fibroblasts into myofibroblasts, disrupting the TGF-β1/Smad2 axis.

Peptide hormones act by binding to receptors on the plasma membrane. They lead to the generation of a second messenger in the cytosol, which changes the activity of an intracellular enzyme, altering the cellular response (Hölscher, 2022). GHRH binds to the splicing variant of the GHRH receptor (SV1), activating Ras, which phosphorylates MAPK (Zhang et al., 2020), stimulating TGF-β expression and activating Smad pathway-dependent α-SMA transcription (Ard et al., 2019). In addition, the peptide hormone GLP-1 has been shown to stimulating α-SMA expression, inhibiting pro-inflammatory pathways. Soluble pro-inflammatory factors such as IL-1 and IFN-γ reduce myofibroblast differentiation (Hinz, 2016). IL-1 induces COX-2 expression and prostraglandin E2 synthesis, activating the transcription of pro-

inflammatory cytokines via NF-kB (Nakano et al., 2020), also inhibiting myofibroblast differentiation. Conversely, IFN-γ reduces myofibroblasts differentiation by activating the repressor protein YB-1, which increases Smad7 gene transcription, a negative regulator of Smad3 signaling (Jacob et al., 2016). Smad7, one of the Smad inhibitors (I-SMADs), is an essential negative regulator of the TGF-β signaling pathway. Smad7 blocks Smad2 phosphorylation and activation, inhibiting the major TGF-β transcriptional pathway (de Ceuninck van Capelle et al., 2020).

4.3 Limitations

Bias analysis showed specific methodological limitations, such as random sequence generation, information on animals' allocation, and outcome assessors blinding; which were underreported in all studies. In addition, information about potential animals' complications, such as death during the experiment, or exclusion for specific reasons were not reported in all studies. However, such limitations do not indicate that these methodological parameters were not investigated in the studies reviewed, aspects that may have been neglected in the preparation of the scientific report. In individual studies, each element of bias was associated with some degree of variability in the research results, with a direct impact on evidence quality. Despite this variability, it was possible to group some studies with similar methods and research outcomes, providing the necessary basis for conducting a meta-analysis. Thus, reliable evidence controlled by specific bias sources was presented, favoring the critical interpretation of the research findings reported in this review.

5. CONCLUSION

In this study, we identified that endocrine stimulation *in vitro* and *in vivo* can have positive or negative effects on myofibroblasts differentiation during skin wounds

healing. In general, testosterone, estrogen, 17-β estradiol, and dexamethasone were effective in stimulating myofibroblast differentiation. GLP-1 and GHRH were effective peptide hormones in positively modulating myofibroblasts differentiation, while this effect was negatively influenced by overexpression of GH. Although TGF-β/Smads is the main pathway activated during myofibroblast differentiation, alternative pathways such as MAPK can activate α-SMA genes expression, regulating fibroblast/myofibroblast dynamics during tissue repair. In addition, the inhibition of myofibroblast differentiation is mainly related to Smad7 activation and the increased release of pro-inflammatory cytokines mediated by NF-κB upregulation. Regarding doses and exposure time, it was observed that 1 to 10 mg/kg estrogen and 2 mg/kg dexamethasone increase myofibroblasts immunostaining 14 days after treatment, while this effect is respectively achieved with 100 nM GLP-1 and 3 mg/kg GHRH 10 to 13 days post-treatment.

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7. SUPPLEMENTARY MATERIAL

Table S1: Search Strategy - Descriptor Filters on Platforms

Data base	Descriptors	Items Found	Time	Date
PUBMED	**#1) Myofibroblasts Filter** "myofibroblasts"[MeSH Terms] OR myofibroblasts [TIAB])	12,432	12:09	08/15/22
	#2) Skin Filter "Skin"[MeSH Terms] OR "Dermis"[MeSH Terms] OR "Granulation Tissue"[MeSH Terms] OR "Epidermis"[MeSH Terms] OR "Keratinocytes"[MeSH Terms] OR "Integumentary System"[MeSH Terms] OR "Dermatology"[MeSH Terms] OR "Dermoscopy"[MeSH Terms] OR "Wounds and Injuries"[MeSH Terms] OR "Fibrosis"[MeSH Terms] OR "Skin injuries"[Title/Abstract] OR "Skin fibrosis"[Title/Abstract] OR "Skin scars"[Title/Abstract] OR "Cicatrix"[MeSH Terms]	1,470,050	12:10	08/15/22
	#3) Hormone filter "steroids"[MeSH Terms] OR "hormones"[MeSH Terms] OR "steroid"[Title/Abstract] OR "steroids"[Title/Abstract] OR "hormone"[Title/Abstract] OR "hormones"[Title/Abstract] OR "anabolic"[Title/Abstract] OR anabolics"[Title/Abstract]	2,061,455	12:10	08/15/22
	#4) **Animal's filter** 1 "animal experimentation"[MeSH Terms] OR "models, animal"[MeSH Terms] OR "invertebrates"[MeSH Terms] OR "Animals"[Mesh:noexp] OR "animal population groups"[MeSH Terms] OR "chordata"[MeSH Terms:noexp] OR "chordata, nonvertebrate"[MeSH Terms] OR "vertebrates"[MeSH Terms:noexp] OR "amphibians"[MeSH Terms] OR "birds"[MeSH Terms] OR "fishes"[MeSH Terms] OR "reptiles"[MeSH Terms] OR "mammals"[MeSH Terms:noexp] OR "primates"[MeSH Terms:noexp] OR "artiodactyla"[MeSH Terms] OR "carnivora"[MeSH Terms] OR "cetacea"[MeSH Terms] OR "chiroptera"[MeSH Terms] OR "elephants"[MeSH Terms] OR "hyraxes"[MeSH Terms] OR "insectivora"[MeSH Terms] OR "lagomorpha"[MeSH Terms] OR "marsupialia"[MeSH	7,201,748	12:11	08/15/22

	Terms] OR "monotremata"[MeSH Terms] OR "perissodactyla"[MeSH Terms] OR "rodentia"[MeSH Terms] OR "scandentia"[MeSH Terms] OR "sirenia"[MeSH Terms] OR "xenarthra"[MeSH Terms] OR "haplorhini"[MeSH Terms:noexp] OR "strepsirhini"[MeSH Terms] OR "platyrrhini"[MeSH Terms] OR "tarsii"[MeSH Terms] OR "catarrhini"[MeSH Terms:noexp] OR "cercopithecidae"[MeSH Terms] OR "hylobatidae"[MeSH Terms] OR "hominidae"[MeSH Terms:noexp] OR "gorilla gorilla"[MeSH Terms] OR "pan paniscus"[MeSH Terms] OR "pan troglodytes"[MeSH Terms] OR "pongo pygmaeus"[MeSH Terms]			
	#5) Animal's filter 2 animals [TIAB] OR animal [TIAB] OR mice [TIAB] OR mus [TIAB] OR mouse [TIAB] OR murine [TIAB] OR woodmouse [TIAB] OR rats [TIAB] OR rat [TIAB] OR murinae [TIAB] OR muridae [TIAB] OR cottonrat [TIAB] OR cottonrats [TIAB] OR hamster [TIAB] OR hamsters [TIAB] OR cricetinae [TIAB] OR rodentia [TIAB] OR rodent [TIAB] OR rodents [TIAB] OR pigs [TIAB] OR pig [TIAB] OR swine [TIAB] OR swines [TIAB] OR piglets [TIAB] OR piglet [TIAB] OR boar [TIAB] OR boars [TIAB] OR "sus scrofa" [TIAB] OR ferrets [TIAB] OR ferret [TIAB] OR polecat [TIAB] OR polecats [TIAB] OR "mustela putorius" [TIAB] OR "guinea pigs" [TIAB] OR "guinea pig" [TIAB] OR cavia [TIAB] OR callithrix [TIAB] OR marmoset [TIAB] OR marmosets [TIAB] OR cebuella [TIAB] OR hapale [TIAB] OR octodon [TIAB] OR chinchilla [TIAB] OR chinchillas [TIAB] OR gerbillinae [TIAB] OR gerbil [TIAB] OR gerbils [TIAB] OR jird [TIAB] OR jirds [TIAB] OR merione [TIAB] OR meriones [TIAB] OR rabbits [TIAB] OR rabbit [TIAB] OR hares [TIAB] OR hare [TIAB] OR diptera [TIAB] OR flies [TIAB] OR fly [TIAB] OR dipteral [TIAB] OR drosphila [TIAB] OR drosophilidae [TIAB] OR cats [TIAB] OR cat [TIAB] OR carus [TIAB] OR felis [TIAB] OR nematoda [TIAB] OR nematode [TIAB] OR nematoda [TIAB] OR nematode [TIAB] OR nematodes [TIAB] OR sipunculida [TIAB] OR dogs [TIAB] OR dog [TIAB] OR canine [TIAB] OR canines [TIAB] OR canis [TIAB] OR sheep [TIAB] OR sheeps [TIAB] OR mouflon [TIAB] OR mouflons [TIAB] OR ovis [TIAB] OR goats [TIAB] OR goat [TIAB] OR capra [TIAB] OR capras [TIAB] OR rupicapra [TIAB] OR chamois [TIAB] OR haplorhini [TIAB] OR monkey [TIAB] OR monkeys [TIAB] OR anthropoidea [TIAB] OR	5,313,112	12:12	08/15/22

	anthropoids [TIAB] OR saguinus [TIAB] OR tamarin [TIAB] OR tamarins [TIAB] OR leontopithecus [TIAB] OR hominidae [TIAB] OR ape [TIAB] OR apes [TIAB] OR pan [TIAB] OR paniscus [TIAB] OR "pan paniscus" [TIAB] OR bonobo [TIAB] OR bonobos [TIAB] OR troglodytes [TIAB] OR "pan troglodytes" [TIAB] OR gibbon [TIAB] OR gibbons [TIAB] OR siamang [TIAB] OR siamangs [TIAB] OR nomascus [TIAB] OR symphalangus [TIAB] OR chimpanzee [TIAB] OR chimpanzees [TIAB] OR prosimians [TIAB] OR "bush baby" [TIAB] OR prosimian [TIAB] OR bush babies [TIAB] OR galagos [TIAB] OR galago [TIAB] OR pongidae [TIAB] OR gorilla [TIAB] OR gorillas [TIAB] OR pongo [TIAB] OR pygmaeus [TIAB] OR "pongo pygmaeus" [TIAB] OR orangutans [TIAB] OR pygmaeus [TIAB] OR lemur [TIAB] OR lemurs [TIAB] OR lemuridae [TIAB] OR horse [TIAB] OR horses [TIAB] OR pongo [TIAB] OR equus [TIAB] OR cow [TIAB] OR calf [TIAB] OR bull [TIAB] OR chicken [TIAB] OR chickens [TIAB] OR gallus [TIAB] OR quail [TIAB] OR bird [TIAB] OR birds [TIAB] OR quails [TIAB] OR poultry [TIAB] OR poultries [TIAB] OR fowl [TIAB] OR fowls [TIAB] OR reptile [TIAB] OR reptilia [TIAB] OR reptiles [TIAB] OR snakes [TIAB] OR snake [TIAB] OR lizard [TIAB] OR lizards [TIAB] OR alligator [TIAB] OR alligators [TIAB] OR crocodile [TIAB] OR crocodiles [TIAB] OR turtle [TIAB] OR turtles [TIAB] OR amphibian [TIAB] OR amphibians [TIAB] OR amphibia [TIAB] OR frog [TIAB] OR frogs [TIAB] OR bombina [TIAB] OR salientia [TIAB] OR toad [TIAB] OR toads [TIAB] OR "epidalea calamita" [TIAB] OR salamander [TIAB] OR salamanders [TIAB] OR eel [TIAB] OR eels [TIAB] OR fish [TIAB] OR fishes [TIAB] OR pisces [TIAB] OR catfish [TIAB] OR catfishes [TIAB] OR siluriformes [TIAB] OR arius [TIAB] OR heteropneustes [TIAB] OR sheatfish [TIAB] OR perch [TIAB] OR perches [TIAB] OR percidae [TIAB] OR perca [TIAB] OR trout [TIAB] OR trouts [TIAB] OR char [TIAB] OR chars [TIAB] OR salvelinus [TIAB] OR "fathead minnow" [TIAB] OR minnow [TIAB] OR cyprinidae [TIAB] OR carps [TIAB] OR carp [TIAB] OR zebrafish [TIAB] OR zebrafishes [TIAB] OR goldfish [TIAB] OR goldfishes [TIAB] OR guppy [TIAB] OR guppies [TIAB] OR chub [TIAB] OR chubs [TIAB] OR tinca [TIAB] OR barbels [TIAB] OR barbus [TIAB] OR pimephales [TIAB] OR promelas [TIAB] OR "poecilia reticulata" [TIAB] OR mullet [TIAB] OR mullets [TIAB] OR seahorse [TIAB] OR seahorses [TIAB] OR mugil curema [TIAB] OR atlantic cod [TIAB] OR shark [TIAB] OR sharks			

	[TIAB] OR catshark [TIAB] OR anguilla [TIAB] OR salmonid [TIAB] OR salmonids [TIAB] OR whitefish [TIAB] OR whitefishes [TIAB] OR salmon [TIAB] OR salmons [TIAB] OR sole [TIAB] OR solea [TIAB] OR "sea lamprey" [TIAB] OR lamprey [TIAB] OR lampreys [TIAB] OR pumpkinseed [TIAB] OR sunfish [TIAB] OR sunfishes [TIAB] OR tilapia [TIAB] OR tilapias [TIAB] OR turbot [TIAB] OR turbots [TIAB] OR flatfish [TIAB] OR flatfishes [TIAB] OR sciuridae [TIAB] OR squirrel [TIAB] OR squirrels [TIAB] OR chipmunk [TIAB] OR chipmunks [TIAB] OR suslik [TIAB] OR susliks [TIAB] OR vole [TIAB] OR voles [TIAB] OR lemming [TIAB] OR lemmings [TIAB] OR muskrat [TIAB] OR muskrats [TIAB] OR lemmus [TIAB] OR otter [TIAB] OR otters [TIAB] OR marten [TIAB] OR martens [TIAB] OR martes [TIAB] OR weasel [TIAB] OR badger [TIAB] OR badgers [TIAB] OR ermine [TIAB] OR mink [TIAB] OR minks [TIAB] OR sable [TIAB] OR sables [TIAB] OR gulo [TIAB] OR gulos [TIAB] OR wolverine [TIAB] OR wolverines [TIAB] OR minks [TIAB] OR mustela [TIAB] OR llama [TIAB] OR llamas [TIAB] OR alpaca [TIAB] OR alpacas [TIAB] OR camelid [TIAB] OR camelids [TIAB] OR guanaco [TIAB] OR guanacos [TIAB] OR chiroptera [TIAB] OR chiropteras [TIAB] OR bat [TIAB] OR bats [TIAB] OR fox [TIAB] OR foxes [TIAB] OR iguana [TIAB] OR iguanas [TIAB] OR xenopus laevis [TIAB] OR parakeet [TIAB] OR parakeets [TIAB] OR parrot [TIAB] OR parrots [TIAB] OR donkey [TIAB] OR donkeys [TIAB] OR mule [TIAB] OR mules [TIAB] OR zebra [TIAB] OR zebras [TIAB] OR shrew [TIAB] OR shrews [TIAB] OR bison [TIAB] OR bisons [TIAB] OR buffalo [TIAB] OR buffaloes [TIAB] OR deer [TIAB] OR deers [TIAB] OR bear [TIAB] OR bears [TIAB] OR panda [TIAB] OR pandas [TIAB] OR "wild hog" [TIAB] OR "wild boar" [TIAB] OR fitchew [TIAB] OR fitch [TIAB] OR beaver [TIAB] OR beavers [TIAB] OR jerboa [TIAB] OR jerboas [TIAB] OR capybara [TIAB] OR capybaras [TIAB])			
	Total manuscripts= (#1 OR #2) AND #3 AND #4 AND #5	**281**	12:13	08/15/22
Data base	**Descriptors**	**Items Found**	**Time**	**Date**
	#1) Myofibroblasts Filter TITLE-ABS-KEY("myofibroblasts")	13,150	12:18	08/15/22

SCOPUS	**#2) Skin Filter** TITLE-ABS-KEY("Skin") OR TITLE-ABS-KEY("Dermis") OR TITLE-ABS-KEY("Granulation Tissue") OR TITLE-ABS-KEY("Epidermis") OR TITLE-ABS-KEY("Keratinocytes") OR TITLE-ABS-KEY("Integumentary System") OR TITLE-ABS-KEY("Dermatology") OR TITLE-ABS-KEY("Dermoscopy") OR TITLE-ABS-KEY("Wounds and Injuries") OR TITLE-ABS-KEY("Fibrosis") OR TITLE-ABS-KEY("Skin injuries") OR TITLE-ABS-KEY("Skin fibrosis") OR TITLE-ABS-KEY("Skin scars") OR TITLE-ABS-KEY("Cicatrix")	1,892,612	12:18	08/15/22
	#3) Hormone filter TITLE-ABS-KEY("nandrolone") OR TITLE-ABS-KEY("steroids") OR TITLE-ABS-KEY("hormones") OR TITLE-ABS-KEY("nandrolone") OR TITLE-ABS-KEY("steroid") OR TITLE-ABS-KEY("steroids") OR TITLE-ABS-KEY("hormone") TITLE-ABS-KEY("hormones") OR TITLE-ABS-KEY("anabolic") OR TITLE-ABS-KEY("anabolics")	1,027,829	12:19	08/15/22
	Total: #1 AND #2 AND #3	**156**	12:20	08/15/22
Data base	**Descriptors**	**Items Found**	**Time**	**Date**
WEB of SCIENCE	**#1) Myofibroblasts Filter** TS=myofibroblasts	12,901	12:25	08/15/22
	#2) Skin Filter TS=Skin OR TS=Dermis OR TS=Granulation tissue OR TS=Epidermis OR TS=Keratinocyte OR TS=Integumentary system OR TS=Dermatology OR TS=Dermoscopy OR TS=Skin wounds OR TS=Skin injuries OR TS=Skin fibrosis OR TS=Skin scar OR TS=Skin cicatrix	779,411	12:26	08/15/22
	#3) Hormone filter TS=nandrolone OR TS=steroids OR TS=hormones OR TS=nandrolone OR TS=steroid OR TS=steroids OR TS=hormone OR TS=hormones OR TS=anabolic TS=anabolics	815,483	12:27	08/15/22
	Total: #1 AND #2 AND #3	**232**	12:27	08/15/22

Table S2: General characteristics of the animal model used in all studies that investigate the endocrine stimuli in the differentiation of fibroblasts into myofibroblasts in the healing process of cutaneous wounds

References	Country	Strain	knockout/transgenic	Sex	Age	Weight	Animals/group
Almeida et al., 2016	Brazil	Swiss mice	-	M	?	25 a 35 g	20
Campbell et al., 2010	England	C57BL/6 mice	Epidermal-specific deletion of ERα and ERβ	F	10 w	?	5 - 7
Dioufa et al., 2010	Greece	C57BL/6 mice	Wild Type/FVB genetic	M	?	?	6
Marchionni et al., 2010	Brazil	Wistar rats	-	M	?	200 a 259g	20
Mukai et al., 2014(A)	Japan	C57BL/6 mice	-	F	8 w	?	21
Mukai et al., 2014(B)	Japan	C57BL/6 mice	-	F	7 w	?	OVX (n=20) and OVX+17b estradiol (n=21)
Mukai et al., 2014(C)	Japan	C57BL/6 mice	-	F	8 w	?	OVX (n=20) and OVX+17b estradiol (n=21)
Romana-Souza et al., 2013	Brazil	Swiss mice	-	M & F	Adult	?	Intact (n=30) and gonadectomized (n =15)
Schurmann et al., 2012	Germany	C57BL/6 mice	C57BL/6J-(ob/ob)	F	12 w	?	10
Gilliver et al., 2005	United Kingdom	Sprague-Dawley(rats)	Smad3 -/-	M	8 w	?	6 - 7

Gilliver et al., 2007	United Kingdom	Sprague-Dawley(rats)	-	M	8 w	?	6 - 7
Gilliver et al., 2009	United Kingdom	Sprague-Dawley (rats)	-	M	8 w	?	6 - 7
Thorey et al., 2004	Germany	C57BL/6 mice.	Phosphoenolpyruvate carboxykinase-bGH transgenic	M & F	?	?	WT F (n=3) WT M (n=2); Transgenic M (n=3) and F (n=2)
Novotny et al., 2011	Slovak Republic	Sprague-Dawley (rats)	-	F	6 w	?	16

M: Male; F: Female; W: week; OVX: Ovarictomized; WT: Wild Type

Table S3: Characteristics of the animal model skin wounds used in all studies that investigate the endocrine stimulus in the differentiation of fibroblasts into myofibroblasts in the healing process of skin wounds

Reference	Lesion	Site	Initial area	Number	Anesthesia	Wound removal (days)
Almeida et al., 2016	E	Dorsal	10mm	1	ketamine & xylazine	04, 07, 14
Campbell et al., 2010	E & I	Dorsal	I - 10mm & E- 6mm	2	?	21
Dioufa et al., 2010	E	Dorsal	4 mm	4	ketamine & xylazine	0, 5, 8,10
Marchionni et al., 2010	E	Dorsal	6 mm	1	Tiletamine chloride	1, 3, 5, 7, 14
Mukai et al., 2014(A)	E	Dorsal	4 mm	2	Pentobarbital	0, 3, 7, 11, 14
Mukai et al., 2014(B)	E	Dorsal	4 mm	2	Pentobarbital	3,7 ,14
Mukai et al., 2014(C)	E	Dorsal	4 mm	2	Pentobarbital	0, 3, 7, 11, 14
Romana-Souza et al., 2013	E	Dorsal	10mm	1	ketamine & xylazine	7 ,14
Schurmann et al., 2012	E	Dorsal	5 mm	6	ketamine & xylazine	1,3,5,7,13
Gilliver et al., 2005	I	Dorsal	10mm	4	Isoflurane	2 ,6
Gilliver et al., 2007	E	Dorsal	10mm	4	Isoflurane	2 ,6

Gilliver et al., 2009	E & I	Dorsal	I - 10mm & E- 6mm	4	Isoflurane	2, 4, 6 ,8
Thorey et al., 2004	E & I	Dorsal	I - 10mm & E- 5mm	4 & 1	ketamine & xylazine	5, 13
Novotny et al., 2011	E & I	Dorsal	I - 10mm & E- 4mm	2	ketamine & xylazine	5, 10

E: Excisional; I: Incisional.

Table S4: Characteristics of the hormones investigated in the differentiation of fibroblasts into myofibroblasts in the healing process of skin wounds.

Reference	Hormone	Quimic Classification	Producer gland	Gonadectomy	Hormones dosage (gonadectomized)	Route	Dose
Almeida et al., 2016	Glucocorticoids (GCs)	Lipid	Adrenal cortex	No	N/A	IP	20 mg/kg
Campbell et al., 2010	17 beta estradiol (17β-E)	Lipid	Ovaries	Ovariectomy	Yes	Sub	0.05mg
Dioufa et al., 2010	Growth hormone-releasing hormone (GHRH)	Peptide	Hypothalamus	No	N/A	Top	100 nM
Marchionni et al., 2010	Dexamethasone (DX)	Peptide	Adrenal cortex	No	N/A	Oral	2 mg/kg
Mukai et al., 2014(A)	17 beta estradiol (17β-E)	Lipid	Ovaries	Ovariectomy	Yes	Top	0.01 g
Mukai et al., 2014(B)	17 beta estradiol (17β-E)	Lipid	Ovaries	Ovariectomy	Yes	Top	0.01 g
Mukai et al., 2014(C)	17 beta estradiol (17β-E)	Lipid	Ovaries	Ovariectomy	Yes	Top	0.01 g

Romana-Souza et al., 2013	Testosterone (Tes) and Estrogen (E)	Lipid	Ovaries/Testis	Castration/Ovariectomy	Yes	N/A	N/A
Schurmann et al., 2012	Glucagon-like peptide (GLP-1)	Peptide	Pancreas (islets)	No	N/A	Oral	3 mg/kg
Gilliver et al., 2005	Dihydrotestosterone (DHT)	Lipid	Testis	Castration	results not shown	Oral	N/A
Gilliver et al., 2007	Dihydrotestosterone (DHT)	Lipid	Testis	Castration	results not shown	N/A	N/A
Gilliver et al., 2009	Dihydrotestosterone (DHT)	Lipid	Testis	Castration	No	Oral	N/A
Thorey et al., 2004	Growth hormone (GH)	Peptide	Pituitary gland	No	N/A	N/A	N/A
Novotny et al., 2011	Estrogen (E)	Lipid	Ovaries	Ovariectomy	No	Sub	1 mg/kg

N/A: Not Applicable, IP: Intraperitoneal; Sub: subcutaneous; Top: Topic

Table S5: General characteristics of in vitro studies that investigated the endocrine stimulus in the differentiation of fibroblasts into myofibroblasts.

Reference	Cells type	Cells lineage	Culture Type	Cell Origen	Culture medium	Day of treatment	Hormone / dose	α-SMA marker
Dioufa et al., 2010	Fibroblasts	Mouse Embryonic	Primary	lung	DMEM	1	GHRH 100 and 500 nM	(+) 100 nM (-) 500 nM
Thorey et al., 2004	Fibroblasts	Mouse Embryonic	immortalized	skin	DMEM	5	GH 12,3/61,3 and 245 ng /ml	(-)

DMEM: Dulbecco's modified Eagle medium