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REVIEW ARTICLE

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Animal experimental research assessing urogynecologic surgical mesh implants: Outcome measures describing the host response, a systematic review and meta-analysis

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Abstract

Aim: Before the introduction of new biomaterials for prolapse surgery, animal studies on the host response are required. Unfortunately, large variation in study design hampers obtaining an overview of the safety and efficacy, and translation to clinical practice. Our aim is to systematically review the literature on all outcome measures describing the host response in animal studies assessing the biocompatibility of urogynecologic surgical mesh implants for prolapse surgery. Furthermore, by meta-analysis, we aim to assess the effect of implantation and compare this to control animals receiving sham surgery or native tissue repair.

Methods: We performed a systematic search from inception to August 2020. Since this is an explorative study we included original, controlled, and non-controlled animal studies describing any host response to the implant. Quantitative outcome measures reported ≥10 times in ≥2 articles were eligible for meta-analysis.

Results: Fifty articles were included in the qualitative synthesis and 36 articles were eligible for meta-analysis. In total, 154 outcome measures were defined and classified into (1) histomorphology, (2) biomechanics and, (3) macroscopic morphology. Animals with vaginal implants demonstrated significantly increased M1 and M2 macrophages, MMP-2, neovascularization, TNF-α, and stiffness, and lower vaginal contractility compared to control animals.

Conclusion: The host response significantly differs in animals after vaginal mesh implantation compared to control animals, both pro- and anti-inflammatory. However, we observed a paucity in the uniformity of reported outcomes. For future animal studies, we propose the development of a core outcome set, which ideally predicts the host response in women.

KEYWORDS

biomaterials, foreign-body reaction, in vivo, pelvic floor, pelvic organ prolapse

1 | INTRODUCTION

Pelvic floor disorders such as urinary incontinence and pelvic organ prolapse (POP) affect many women, with the incidence increasing up to 50% with age.[1] Unfortunately, long-term results of native tissue repair (NTR) are far from optimal and reoperation rates for recurrent prolapse symptoms are as high as 17%–29%.[2,3] High-failure rates of NTR might result from the use of the patient's own—already defective—connective tissue to restore the support system. Surgical meshes were introduced to improve the outcome of POP surgery by providing durable mechanical support. However, synthetic implants might cause a persistent chronic and uncontrolled inflammatory response and this may result in fibrosis and complications as implant exposure and pain.[4] This has led to an evolvement to lightweight, monofilament and macroporous implants which induce a milder host response.[5] Nonetheless, for years the FDA is warning about potential risks of pelvic floor implants and in 2019 the FDA ordered manufacturers of transvaginal meshes to stop selling their devices because of insufficient effectiveness and non-reassuring safety of the mesh.[6,7]

Until today, researchers are pursuing to develop a pelvic floor implant that gives lasting restoration of the anatomy but causes minimal side effects. As opposed to the earlier, before the introduction of new biomaterials for pelvic floor surgery, animal studies on the host response have become a requirement to assess the safety and efficacy in preparation of clinical studies.[8] The host response is the reaction of the body to the presence of a material and begins immediately upon implantation, but will last a lifetime and it is decisive in determining the success in the long term. It is defined by the response to tissue injury during implantation and the response evoked by the biomaterial itself.[9] The host response towards these implants is essential for the development of new load-bearing tissue, but if being uncontrolled it can cause adverse events.[4] The host response is not a single well-defined outcome measure but consists of a sequence of host reactions including tissue injury, acute inflammation, chronic inflammation, and wound healing, along with a myriad of different cell types and mediators.[9] Ideally, we would like to know which animal models and outcome measures predict the host response and subsequent success of implants in women. However, various animal models have been used and various outcome measures have been reported. The large variation in study design results in difficulties to aggregate, interpret, and generalize the results and challenges translation to clinical practice. Therefore, we first aim to give an overview of all outcome measures describing the host response in animal studies assessing urogynecologic surgical mesh implants for prolapse surgery. In addition, using meta-analysis we aim to quantitatively assess the effect of mesh implantation compared to NTR or sham surgery and investigate the influence of certain characteristics, such as species and type of implant. Finally, we aim to give insight into differences in the host response between different implantation sites in a second meta-analysis. The overall objective of this systematic review is to eventually improve the interpretation of in vivo studies and give researchers considerations for future study design.

2 | MATERIALS AND METHODS

This systematic review is adherent to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.[10] The protocol was registered on the international prospective register of systematic reviews (PROSPERO) on August 9, 2019, under registration number CRD42019142850. Detailed methodological information can be found in Supplementary file 1.

2.1 | Literature search

A medical information specialist (Jacqueline Limpens) performed a systematic search in Ovid MEDLINE and Ovid EMBASE from inception onwards (with the last update on August 14, 2020) using controlled terms (i.e,. MeSH-terms in MEDLINE) as well as free text terms for (1) POP, pelvic floor, or vaginal reconstruction and (2) various implant terms combined with (3) an animal search filter (Supplementary file 2). Conference abstracts were excluded in EMBASE. No further restrictions were applied. We crosschecked reference lists and the citing articles of included papers and relevant reviews for additional relevant studies using Web of Science. The records retrieved were imported and de-duplicated in EndNote X9.

2.2 | Study selection

Titles and abstracts were independently screened by two reviewers (Kim W. J. Verhorstert and Brita S. Kortz) in Early Review Organizing Software (www.eros-systemtic-review.org) using the following exclusion criteria (1) no primary article, (2) no animal experiment, (3) no implant indicated for POP and (4) no vaginal implantation. Only original, controlled, or noncontrolled studies were eligible when animals received a vaginal implant,

concomitant abdominal or subcutaneous implantation was permitted. In the second round of screening in Rayyan,[11] full texts were screened using the same exclusion criteria as described above and additionally (5) no outcome measure describing host response. Since this is an explorative study, no further restrictions were made and the host response included any reaction to the implant, both direct (e.g., histological) and indirect outcomes (e.g., macroscopic observations) of the host response. Although strictly speaking NTR or sham surgery would not provoke a host response since there is no insertion of a foreign body, we also evaluated these outcome measures in control animals to make a comparison to animals with implants possible. Any discrepancies between the two reviewers were resolved by discussion, where necessary, a senior reviewer (Zeliha Guler) was consulted.

2.3 | Study characteristics

From all included articles we extracted bibliographical information (author, year) and various study design and animal model characteristics (species, intervention, type of implant(s), method of insertion, and duration of follow-up (Supplementary file 3)). Next, all outcome measures reported describing the host response were extracted. Due to the wide variety in outcome measures, we classified them into three major groups (1) histomorphology (including histology, immunohistochemistry, and biochemistry), (2) biomechanics (active and passive), and (3) macroscopic morphology, and registered whether the outcome was reported qualitative or quantitative.

2.4 | Extraction outcome data

Outcome data were extracted in duplicate by two independent reviewers (Kim W. J. Verhorstert and Aksel N. Gudde). The frequency of all reported outcome measures was calculated. The effect of mesh surgery on macroscopic morphology could be analyzed without a control group, since macroscopic morphological changes (e.g., exposures) only occur in implanted animals. Other outcome measures needed to have an appropriate control group to be eligible for meta-analysis (sham surgery or NTR), to ensure possible interpretation of the results (e.g., histological scoring). If a study reported data from several experimental groups, this was considered as separate comparisons. In case of missing data, the authors were contacted. When medians and interquartile ranges were reported, these were converted to means and standard deviations (*SD*) as reported by Wan et al.[12]

2.5 | Quality assessment

We performed a risk of bias assessment for all studies with an appropriate control group using the SYRCLE Risk of Bias tool.[13] Because reporting of experimental details on animals, methods, and materials is often very poor, we added two items on reporting: reporting of any measure of randomization and blinding, to overcome the problem of judging too many items as "unclear risk of bias."[14] The quality of all included articles was independently scored by two reviewers (Kim W. J. Verhorstert and Brita S. Kortz), any discrepancies were solved by discussion.

2.6 | Meta-analysis

Meta-analysis was performed in Comprehensive Meta-Analysis (CMA) software (version 3.0). Quantitative outcome measures reported ≥10 times in ≥2 articles were eligible for meta-analysis. If the same group was used for multiple comparisons, the number of animals was divided by the number of comparisons. In case the animal number was not an integer, it was rounded to the nearest whole number. In some groups, the *SD* was 0 and we inferred the *SD* based on similar other groups within the same study. If a study reported dichotomous outcomes, but all other studies reported continuous data (e.g., contraction) these results were excluded for meta-analysis.

Depending on the type of data, results were reported as odds ratio (OR) and Hedges *g* (histomorphology and biomechanics) or event rate and mean (macroscopic morphology), all with their 95% confidence intervals (CIs). We used the random effects model, which takes into account the precision of individual studies and the variation between studies and weights each study accordingly. I^2 was used to determine the level of between-study heterogeneity. In the first meta-analysis, we compared animals with vaginal implants with control animals, and in the second meta-analysis, we compared vaginal implants with abdominal implants.

Predefined subgroup analyses were planned for species, type of implant, time point, and method of implantation (transvaginal or transabdominal) and were only conducted in case ≥3 independent comparisons were available from ≥3 articles. We expected the variance to be comparable within the subgroups; therefore, a common among-study variance across subgroups was assumed. The *p*-value was adjusted according to the Bonferroni method to account for multiple testing ($p \times$ number of comparisons).

Sensitivity analyses were conducted to determine the robustness of our findings (additional methodological information on subgroup and sensitivity analyses: Supplementary file 1). No assessment for publication bias has been performed due to the limited number of comparisons per outcome measure.

3 | RESULTS

3.1 | Literature search results

The search identified 399 unique references (Figure 1), of which 290 could be excluded based on title and abstract. Out of the remaining 109 articles (27%), full texts were retrieved to assess eligibility. Eventually, 50 articles could be included in the qualitative synthesis and 36 articles were eligible for meta-analysis (Supplementary file 4).

3.2 | Study characteristics and outcome measures

Rabbits (31%) were the most used species, followed by rats (29%), sheep (24%), macaques (14%), and dogs (2%). Regarding the type of implant, polypropylene implants (39%) and polypropylene hybrid implants (28%) were mostly used. The timing of outcome assessment was generally more than 28 days (66%) and in 71% the method of implantation was transvaginal (Figure 2).

Out of the 50 included articles in this systematic review, only 20 articles (40%) used a sham surgery or NTR control. In total, 154 unique outcome measures describing the host response were identified (Table 1A). Of these outcome measures, 101/154 (66%) were quantitative and assessed against an appropriate control group (Table 1B). However, only 17 of these outcomes were eligible for our meta-analysis based on the frequency of reporting: apoptosis, elastin, M1-macrophages, M2-macrophages, matrix-metalloproteinase 2 (MMP-2), neovascularization, smooth muscle, tumor necrosis factor-alpha (TNF-α), total collagen, contractility, stiffness, contraction, degradation, erosion, exposure, extrusion, and implant retrieval.

3.3 | Quality assessment

In general, the majority of items assessed in the risk of bias analysis showed an unclear risk of bias due to insufficient reporting of essential methodological details (Figure 3). Although 63% of the articles stated any form of randomization, in none of the included references the allocation sequence was adequately described. Blinding at any level was described in 42% of the articles, however, the allocation was adequately concealed in only 21% and none of the studies reported blinding of research staff during the course of the experiments (performance bias). Regarding blinding of the outcome assessor, for histomorphology, this was adequately performed and reported in only 20%, but not reported in most other cases.

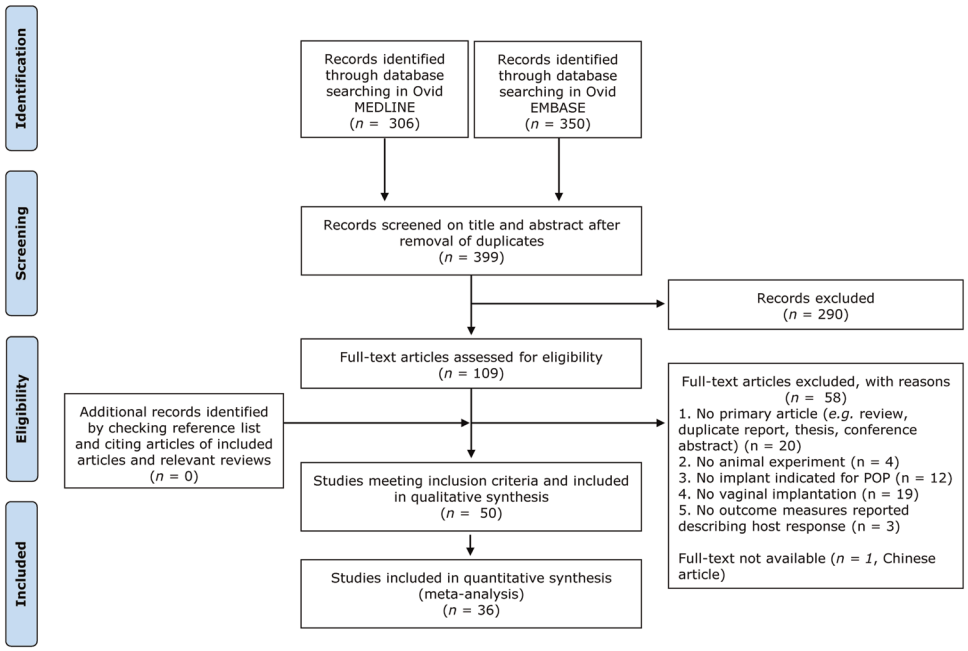

FIGURE 1 PRISMA flowchart of search and screening process

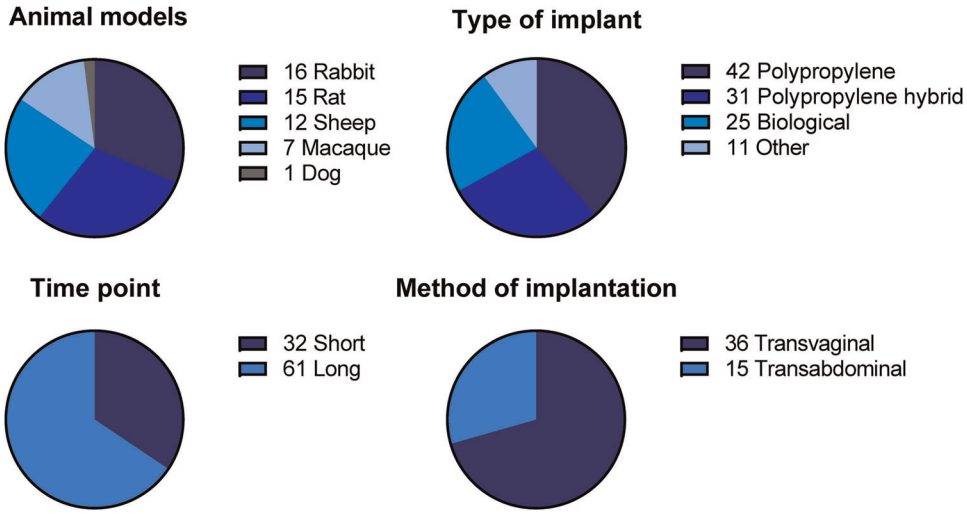

FIGURE 2 Descriptive characteristics of all included articles. Some articles included multiple animal models, type of implants, time points, or methods of implantations; therefore, the total number exceeds the 50 articles

3.4 | Meta-analysis

3.4.1 | Histomorphology and biomechanics in controlled animal studies

Animals with vaginal implants demonstrated a significant increase in M1-macrophages (Hedges $g = 1.85$ [0.83–2.88]), M2-macrophages (Hedges $g = 2.74$ [1.83–3.65]), MMP-2 (Hedges $g = 2.80$ [1.82–3.78]), neovascularization (Hedges $g = 1.17$ [0.84–1.50]), and TNF-α (Hedges $g = 0.83$ [0.11–1.56]) compared to control animals (Table 2A). Furthermore, animals with vaginal implants had significantly lower tissue contractility (Hedges $g = -0.55$ [−0.97 to −0.13]) and higher stiffness values (Hedges $g = 0.68$ [0.20–1.17]) compared to control animals. For apoptosis, elastin, smooth muscle, and total collagen amount, no significant differences were observed. From all outcome measures, subgroup analyses could be performed for neovascularization, total collagen, contractility, and stiffness. Subgroup analyses revealed no significant differences in neovascularization between polypropylene and polypropylene hybrid implants, in total collagen between polypropylene hybrid and biological implants or in stiffness between ewes and macaques or transvaginal and transabdominal implantations. However, vaginal contractility was significantly more decreased after transabdominal implantation (Hedges $g = -1.27$ [−1.77 to −0.77]) compared to transvaginal implantation (Hedges $g = 0.05$ [−0.41 to 0.50]; $p < 0.01$). Other predefined subgroups were too small for meaningful analyses (Supplementary file 5).

3.4.2 | Macroscopic morphology in all animals with implants

Overall, there was 32.7% contraction [27.8–37.7] and subgroup analysis showed no significant differences in contraction between polypropylene and polypropylene hybrid implants (Table 2B). In one article, a group showed "too much contraction to measure," and provided no absolute data.[15] Since leaving this data out would provide an underestimation, it was decided to use the highest mean contraction percentage and *SD* from all included interventions (61.2 ± 17.3). During a sensitivity analysis, this data was left out and the overall contraction was still 31.8% [26.9–36.7].

The erosion rate was 11.6% [6.8–19.0] and subgroup analysis revealed no significant differences in erosion rates between short or long follow-up, nor after a sensitivity analysis changing the definitions of follow-up (as described in Supplementary file 1). While the overall vaginal exposure rate was 20.1% [16.8–24.0], vaginal exposures were less common in rats (10.5% [6.9–15.5]) compared to ewes (25.5% [19.6–32.3]; $p < 0.01$) and rabbits (23.0% [16.7–30.8]; $p = 0.03$).

3.4.3 | Comparison of vaginal and abdominal wall implantations

In the second meta-analysis, we compared vaginal and abdominal wall implantations in the same animal, five studies reported the results of contraction on both sites (11 independent comparisons, 72 vaginal and 83 abdominal implants). Contraction in vaginally implanted

TABLE 1A All qualitative and quantitative outcome measures reported in the included studies, categorized in histomorphology, biomechanics, and macroscopic morphology

Histomorphology		Biomechanics	Macroscopic morphology
Apoptosis	Leukocytes	Break strength	Abscess, deep
Arginase	Lymphocytes	Comfort zone length	Abscess, subcutaneous
Calcification	Lymphocytes, B	Contractility	Adhesions
Cell proliferation	Lymphocytes, T	Elastic modulus	Angiogenesis
Cellular infiltration	M1 macrophages	Electrical field stimulation	Color
Cellular/collagen ratio	M2 macrophages	Energy absorbed	Contraction
Collagen alignment	M2/M1 ratio	Final elongation percentage against force	Degradation
Collagen composition	Macrophages	Leak point pressure	Dehiscence
Collagen degradation	Mast cells	Length at break point	Ellipticity
Collagen density	Mesh integration	Maximum elongation	Encapsulation
Collagen, total	MMP1	Nerve mediated contraction	Erosion
Collagen I	MMP2	Receptor-mediated contraction	Exposure
Collagen III	MMP8	Stiffness	Exposure suture
Collagen III/I	MMP9	Tensile strength	Extrusion
Collagen, immature/disorganized	MMP13	Tissue mesh detachment strength	Fibrosis
Collagen, mature/organized	Monocytes	Ultimate load	Fluid collection
Collagenase activity	Mononuclear cells	Strain or load at failure	Folding
Collagen organization	Muscle penetration	Voiding interval	Formation of tissue bands
Connective tissue	Myocytes	Voiding pressure	Hematoma
Degeneration	Myofibroblasts	Voiding volume	Incorporation
Delineation of layers	Neovascularization		Induration
Disruption	Nerve density, adrenergic		Infection, local
ECM gene expression, COL1a	Nerve density, cholinergic		Infection, systemic
ECM gene expression, COL3a	Nerve density, peripheral		Palpability of material
ECM gene expression, ELN	Nerve growth factor		POP-Q assessment
ECM gene expression, FBN5	Neuronal network		Prominence
Elastin	Neutrophilic cells		Retrieval of implant
Elastin degradation	NO synthetase		Separation
Eosinophils	Plasma cells		Support
Epithelial thickness	Polymorphonuclear cells		Thickness (mesh-tissue complex)
Epithelial trapping	Rejection		Topology
Epithelization	Smooth muscle		
Epitheloid cells	Smooth muscle bundle size		
Fibrin	Smooth muscle organization		
Fibroblastic proliferation	Smooth muscle thickness		
Fibroblasts	Smoothelin		
Fibrocytes	Sub-epitelium		
Foreign body giant cells	Surface between epithelium and implant		
Foreign body reaction	T-cells, CD4		
GAG	T-cells, CD8		
Granulocytes	Th1/Th2 ratio		
Hyperplastic tissue	Th-1-cells		
IFN-y	Th-2-cells		
IL-1	Tissue ingrowth		
IL-4	Tissular colonization		
IL-6	TNF-α		
IL-10	Tropoelastin		
IL10+IL4/TNF-α+IL12	Tropoelastin degradation		
IL-12	Ulceration		
IL-β	Vaginal thickness		
Immune response			
Inflammation			
Integration			

TABLE 1B All quantitative outcome measures reported in the studies with appropriate control group, categorized in histomorphology, biomechanics, and macroscopic morphology

Histomorphology		Biomechanics	Macroscopic morphology
Apoptosis	Lymphocytes	Comfort zone length	Abscess, deep
Cell proliferation	Lymphocytes, B	Contractility	Abscess, subcutaneous
Cellular/collagen ratio	Lymphocytes, T	Electrical field stimulation	Adhesions
Collagen degradation	Macrophages	Energy absorbed	Contraction
Collagen density	M1 macrophages	Final elongation percentage against force	Degradation
Collagenase activity	M2 macrophages	Leak point pressure	Dehiscence
Collagen, total	M2/M1 ratio	Length at break point	Ellipticity
Collagen, immature/disorganized	Mast cells	Maximum elongation	Exposure
Collagen, mature/organized	MMP1	Nerve mediated contraction	Exposure suture
Collagen I	MMP2	Receptor-mediated contraction	Fluid collection
Collagen III	MMP8	Stiffness	Folding
Collagen III/I	MMP9	Tensile strength	Incorporation
Connective tissue	MMP13	Ultimate load	Infection, local
Disruption	Muscle penetration	Voiding interval	POP-Q assessment
ECM gene expression, COL1a	Myocytes	Voiding pressure	Prominence
ECM gene expression, COL3a	Myofibroblasts	Voiding volume	Separation
ECM gene expression, ELN	Neovascularization		Thickness mesh-tissue complex
ECM gene expression, FBN5	Nerve density, adrenergic		Topology
Elastin	Nerve density, cholinergic		
Elastin degradation	Nerve density, peripheral		
Fibroblastic proliferation	Nerve growth factor		
Fibroblasts	Neuronal network		
Foreign body giant cells	Polymorphonuclear cells		
Foreign body reaction	Smooth muscle bundle size		
GAG	Smooth muscle thickness		
Granulocytes	Smoothelin		
IL-1	Sub-epithelium thickness		
IL-4	T-cells, CD4		
IL-10	T-cells, CD8		
IL10+IL4/TNF-α+IL12	Th-1-cells		
IL-12	Th-2-cells		
Inflammation	TNF-α		
Leukocytes	Tropoelastin		
	Tropoelastin degradation		

animals was significantly higher than abdominally implanted animals (Hedges $g = 2.16$ [1.66–2.67], $I^2 = 40\%$), even after a sensitivity analysis leaving "too much contraction to measure" data out (Hedges $g = 2.04$ [1.56–2.52], $I^2 = 30\%$) as described above. Seven articles reported exposures of both vaginal and abdominal implants (23 independent comparisons, 163 vaginal and 187 abdominal implants), and exposures were significantly more common in the vagina compared to the abdominal wall (OR = 3.44 [1.61–7.35], $I^2 = 0\%$).

4 | DISCUSSION

This systematic review identified 154 unique outcome measures in 50 articles describing the host response in animal experimental research assessing the biocompatibility of urogynecologic surgical mesh implants. Outcome measures investigated were classified as histomorphologic outcome measures ($n = 103$) including histology and biochemistry, or macroscopic morphologic ($n = 31$) or biomechanical ($n = 20$) outcomes. Meta-analysis could only include 11% of the outcomes due to the infrequent and qualitative nature of outcome reporting. We, therefore, conclude that animal studies on host response after vaginal mesh surgery are highly heterogeneous, confirming previous observations in narrative reviews.[16,17]

Our meta-analysis revealed significant differences, both pro- and anti-inflammatory, in host response after vaginal mesh implantation compared to control animals. During a controlled host response, the formation of new tissue and subsequent wound healing is expressed by an increase in neovascularization and this was significantly higher in implanted animals. M1 and M2 macrophages,

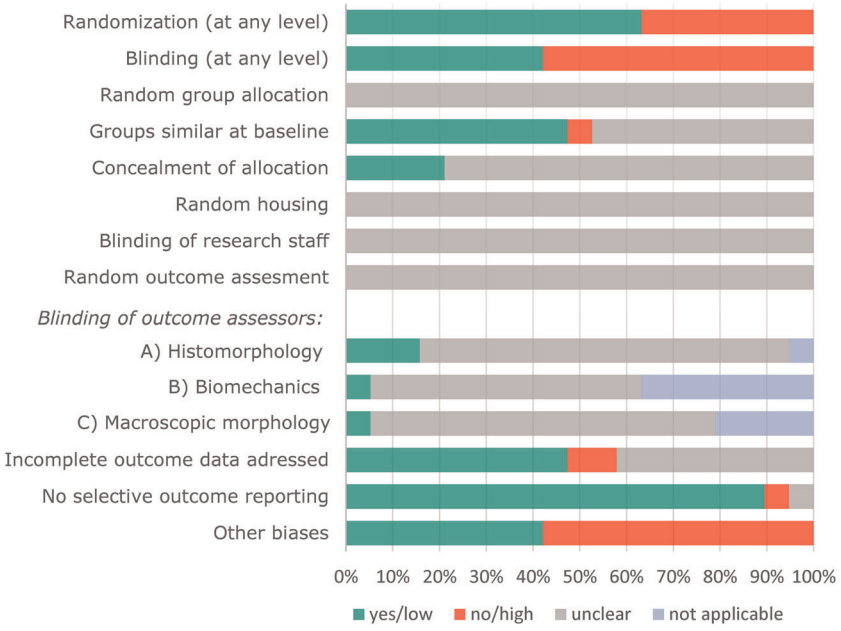

FIGURE 3 Quality assessment of studies with an appropriate control group. For "randomization" and "blinding" a "yes" score indicates "reported," and a "no" score indicates "unreported." For other item a "yes" score indicates low risk of bias; a "no" score indicates high risk of bias; and a "?" score indicates unknown risk of bias

MMP-2 and TNF-α were also significantly increased after vaginal mesh implantation compared to control animals. TNF-α is a pro-inflammatory cytokine, can be secreted by macrophages and plays together with other cytokines an important role in the early inflammatory response.[18] MMP-2 is a proteolytic enzyme capable of degrading and digesting components within the extracellular matrix and important proteins like collagen and elastin.[19,20] In vaginal tissue of women with mesh complications, TNF-α, MMP-2, and pro-inflammatory M1 macrophages have shown to be significantly higher compared to tissue of women without a mesh,[21] as an expression of impaired wound healing. Although in these women macrophages were predominantly of the pro-inflammatory M1 phenotype, as seen in our meta-analysis of animal studies, also anti-inflammatory M2 macrophages were significantly increased,[21] presumably as an expression of constructive remodeling.[22]

Regarding the biomechanical outcomes, we observed a significant increase in vaginal stiffness and a decrease in vaginal contractility, indicating the possible negative effect of mesh implantation on the vaginal wall functionality. Whereas a certain degree of stiffness is required for load-bearing capacity, too high stiffness can cause an impairment in the normal functioning of the vagina.[23,24] Furthermore, while during a controlled host response the contractile function of the vagina is maintained, vaginal contractility can be altered in the presence of implants due to fibrosis, or a decreased collagen and elastin content and is an expression of smooth muscle functioning.[23,25]

Meta-analysis showed an overall exposure rate of 20.1%, but the incidence of vaginal exposures differed hugely among studies. While in many studies no exposures were observed, in others over half of the animals developed a vaginal exposure. Subsequently, subgroup analysis revealed that exposures were significantly more common in ewes and rabbits compared to rats. Larger implants were used in sheep and rabbits and these cause a larger mesh burden and have shown to be a risk factor for vaginal exposures[26] which is in line with observations in women.[27,28] Although the implants in rats were smaller, the rat also has a smaller vagina, but this could also have led to an underestimation in the observation of exposures due to the limited view. Exposure rates in women are lower, approximately around 12%.[29] The higher rate in animals, could be explained for various reasons, such as the different vaginal environment, the experience of the surgeon in the technique, and possibly the use of more experimental types of implants in animal studies.

When comparing vaginal and abdominal implantations, we observed significantly more exposures and a higher contraction rate in the vagina compared to the abdominal wall. The vagina has a different microflora and increased vascularization compared to the abdominal wall, which may cause differences in the local host response.[30]

4.1 | Strengths and limitations

To our knowledge, this is the first systematic review on all outcome measures describing the host response in animal experimental research on urogynecologic surgical mesh implants and assessing the effect of mesh implantation by meta-analysis. Further strengths of this

TABLE 2A Histomorphologic and biomechanical outcomes meta-analysis

Outcome measure	Hedges g	95% CI (*p*-value for subgroups)	I^2	No. of comparisons	No. of articles	No. of animals	Unit
Apoptosis	0.02	−0.44 to 0.48	11.4%	10	4	106	% apoptotic cells
Elastin	0.18	−0.67 to 1.02	71.5%	12	4	117	Concentration, normalized values, % dry weight or % positive cells
M1 macrophages	1.85	0.83 to 2.88[a]	76.8%	13	3	106	Histologic scoring or % positive cells
M2 macrophages	2.74	1.83 to 3.65[a]	64.4%	13	3	106	Histologic scoring or % positive cells
MMP-2	2.80	1.82 to 3.78[a]	74.9%	16	4	150	Density/relative expression or normalized values
Neovascularization – PP vs. PP hybrid	1.17 1.08 vs. 1.96	0.84 to 1.50[a] NS	7.5%	22	5	176	Histologic scoring or density/relative expression
Smooth muscle	0.20	−0.42 to 0.82	63.2%	15	5	147	Histologic scoring or smooth muscle thickness
TNF-α	0.83	0.11 to 1.56[a]	68.5%	13	3	117	Concentration or density/relative expression
Total collagen – PP hybrid vs. biological	0.17 0.61 vs. −0.25	−0.62 to 0.95 NS	67.8%	12	4	117	Histologic scoring or % dry weight
Contractility – TA vs. TV	−0.55 −1.27 vs. 0.05	−0.97 to −0.13[a] $p < 0.01$	34.3%	17	6	156	mN, mN/mm^3, or mN/g
Stiffness – Ewe vs. Macaque – TA vs. TV	0.68 1.06 vs. 0.14 0.35 vs. 0.88	0.20 to 1.17[a] NS NS	52.0%	20	6	173	N/mm

Note: Only subgroup analysis is shown which meets the requirements for subgroup analysis: ≥3 comparisons from ≥3 articles. See Supplementary file 4 for all subgroups per outcome measure, including CI of the above subgroup analysis.

Abbreviations: CI, confidence interval; I^2, heterogeneity; MMP-2, matrix metalloproteinase-2; N, number; NS, nonsignificant; PP, polypropylene; TA, transabdominal; TNF-α, tumor necrosis factor-alpha; TV, transvaginal; vs., versus.

[a]Significant difference between animals with vaginal implants and control animals.

TABLE 2B Macroscopic morphology outcomes meta-analysis

Outcome measure	Event rate/mean	95% CI	I^2	No. of comparisons	No. of articles	No. of animals
Contraction	32.7%[a]	27.8 to 37.7	90.1%	29	8	174
- PP vs. PP hybrid	30.3% vs. 32.1%	NS				
Sensitivity analysis	*31.8%*	*26.9 to 36.7*				
Degradation	40.1%[b]	23.9 to 58.8	36.5%	15	5	68
Erosion	11.6%[b]	6.8 to 19.0	0%	24	4	95
- Short vs. long	12.1% vs. 11.1%	NS				
Exposure	20.1%[b]	16.8 to 24.0	0%	114	25	584
- Ewe vs. rabbit	25.5% vs. 23.0%	NS				
- Rabbit vs. rat	23.0% vs. 10.5%	$p = 0.03$				
- Ewe vs. rat	25.5% vs. 10.5%	$p < 0.01$				
- PP vs. PP hybrid	24.2% vs. 19.3%	NS				
- PP vs. biological	24.2% vs. 14.3%	NS				
- PP vs. other	24.2% vs. 16.1%	NS				
- PP hybrid vs. biological	19.3% vs. 14.3%	NS				
- PP hybrid vs. other	19.3% vs. 16.1%	NS				
- biological vs. other	14.3% vs. 16.1%	NS				
- Short vs. long	17.1% vs. 21.2%	NS				
- TA vs. TV	16.8% vs. 20.4%	NS				
Extrusion	26.0%[b]	13.9 to 43.3	27.3%	13	3	65
Implant retrieval	75.3%[b]	65.0 to 83.3	13.6%	17	5	133
- PP vs. biological	85.3% vs. 70.4%	NS				

Note: Only subgroup analysis is shown which meets the requirements for subgroup analysis: ≥3 comparisons from ≥3 articles. See Supplementary file 4 for all subgroups per outcome measure, including CI of the above subgroup analysis.

Abbreviations: CI, confidence interval; I^2, heterogeneity; N, number; NS, nonsignificant; PP, polypropylene; TA, transabdominal; TV, transvaginal; vs., versus.

[a]Mean value.

[b]Event rate.

review are the broad search, and ensuring methodological quality by a collaboration with the SYstematic Review Center for Laboratory animal Experimentation (SYRCLE). Although we observed a moderate level of between-study heterogeneity for most outcome measures, exploring this heterogeneity is one of the added values and might help to inform the design of future studies. Unfortunately, our planned subgroup analyses contained often too few comparisons to conduct meaningful analyses (Supplementary file 5). Nevertheless, to account for anticipated heterogeneity, we used a random rather than fixed-effects meta-analysis.

However, this review has some limitations. Since the etiology of mesh complications is a multifactorial process in women,[31] certain outcomes may not solely be the result of the host response elicited by the implant. This is one of the limitations of the translation of these animal studies to clinical practice. Furthermore, the large variety in outcome measures and the lack of an appropriate control group in the majority of included articles, hampered meta-analysis of possibly relevant outcomes (e.g., infection, inflammation, or fibroblastic proliferation). However, we observed a trend over the years towards more studies including a sham surgery or NTR group (Supplementary file 6). In addition, the risk of bias could not be estimated for the majority of the studies due to the lack of reporting certain essential methodological details. Although this is common for animal studies, it may influence the results and conclusions drawn. This review also suffers from indirectness issues[32] since most animals in this systematic review did not have clinical signs of prolapse and were not postmenopausal, as most human patients are, and this may have an effect on the local host response and wound healing.[33] However, increased TNF-α, MMP-2, and M1- and M2-macrophages after mesh implantation were seen both in women[21] as the animals in this review. Finally, the estimated effects in this review may be inflated as a consequence of publication bias. Unfortunately, we could not assess publication bias due to too limited number of studies.

4.2 | Implications

We suggest for future studies to include an appropriate control group and focus on reporting all important

methodological details using guidelines.[34,35] Items such as randomization, sample size calculation, and blinding are key aspects in ensuring rigorous and reproducible animal research. Furthermore, we suggest developing a core outcome set of quantitative outcome measures including direct (e.g., histology) and indirect (e.g., macroscopic observations and biomechanics) representatives for the host response. The host response should be evaluated in the short and long term. In our opinion, the direct outcome measures of the host response should include cell types and mediators of acute (e.g., neutrophils) and chronic inflammation (e.g. macrophages), and subsequent wound healing with granulation tissue development (e.g., neovascularization, fibroblasts, and connective tissue formation), the foreign body reaction (e.g., foreign body giant cells) and fibrous capsule formation (e.g., collagen and elastin deposition). Based on our systematic review and previous studies, animal experiments demonstrating (1) non or limited macroscopic changes such as exposure or contraction, (2) an improved tissue regeneration indicated by an M2 response and neovascularization, and (3) improved tissue biomechanics without fibrotic tissue formation which may be represented by a change in tissue stiffness by the contribution of collagen, elastin and MMP activity without or limited decline in contractility; may demonstrate the minimal requirements to assess the performance of the urogynecologic surgical mesh implants. However, this core outcome set should ideally be designed and scaled up/down by experts in the field, including (uro) gynecologists, animal ethics, and scientists with expertise in animal experimental research, histology, biochemistry, and/or biomechanics. Besides as demonstrated by meta-analysis, the host response in the vagina differs from the abdomen. For this reason, we believe that future in vivo studies on new biomaterials for POP should mainly focus on vaginal implantations. Yet for some specific research questions, a smaller animal model might be more suitable. Most challenging, but of high relevance and necessity to study, these outcome measures should predict the host response in women and the possible development of local adverse events. Although no single animal model is able to mimic all human aspects, using this core set of outcome measures improves interpretation, aggregation, and translation of results and probably makes animal experimental research more effective.

5 | CONCLUSIONS

Animals with vaginal implants show a significant increase in M1 and M2 macrophages, MMP-2, neovascularization, TNF-α and stiffness, and lower vaginal contractility compared to control animals receiving sham surgery or NTR. Furthermore, implant exposure and contraction were significantly higher in the vagina as compared to the abdominal wall. However, we observed a large variety in outcome measures used in this type of research and consequently meta-analysis could only be performed for 11% of the outcomes due to the insufficient quality and incompleteness of reported outcomes. Finally, we would like to address the urge for animal experimental research using appropriate control groups, rigorous reporting of all essential methodological details, and inclusion of standardized quantitative outcome measures representing the different phases of the host response, to eventually improve the translation of animal experimental research to clinical practice.

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CONFLICT OF INTERESTS

Ms. Verhorstert reports grants from ZonMw, during the conduct of the study. Mr. Gudde has nothing to disclose. Ms. Kortz has nothing to disclose. Dr. Limpens has nothing to disclose. Dr. Roovers reports grants from ZonMw, during the conduct of the study; grants from Tepha, grants from Urogyn, grants from Coloplast, outside the submitted work. Dr. Hooijmans reports grants from ZonMw, during the conduct of the study. Dr. Guler has nothing to disclose.

AUTHOR CONTRIBUTIONS

Kim W. J. Verhorstert: Conceptualization, formal analysis (lead), funding acquisition, investigation, methodology, writing – original draft preparation, and writing – review & editing. **Aksel N. Gudde**: Investigation and writing – review & editing. **Brita S. Kortz**: Investigation and writing – review & editing. **Jacqueline Limpens**: Investigation and writing – review & editing. **Jan-Paul W. R. Roovers**: Conceptualization, supervision, and writing – review & editing. **Carlijn R. Hooijmans**: Formal analysis, methodology, supervision, and writing – review & editing. **Zeliha Guler**: Conceptualization, funding acquisition, supervision, and writing – review & editing.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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