ISSN 0022-0930, Journal of Evolutionary Biochemistry and Physiology, 2025, Vol. 61, No. 2, pp. 551–560. © Pleiades Publishing, Ltd., 2025.

EXPERIMENTAL PAPERS

# Sex-Specific Peculiarities of Clinical Manifestations of Inflammatory Bowel Diseases in Cohoused *Muc2* Knockout Mouse Siblings

**K. S. Milutinovich[a],* and V. S. Popov[a]**

[a] *Lomonosov Moscow State University, Moscow, Russia*
**e-mail: Milutinovic.Ksenija@yandex.ru*

Received January 13, 2025; revised March 14, 2025; accepted March 20, 2025

**Abstract**—Clinical symptoms of inflammatory bowel diseases (IBD) are the key indicators of disease severity. A clear understanding of the variability in the manifestation of clinical symptoms and their timing is crucial for tracking the effects of treatments on the course of IBD. One of the effective IBD models is the *Mucin 2* knockout (*Muc2*–/–) mouse strain, which exhibits clinical symptoms similar to those in humans. Here, we investigated the sex-specific manifestation of clinical symptoms in *Muc2*–/– mice to develop a protocol for evaluating the effects of drugs on disease progression and severity in this model. The study assessed the degree of rectal prolapse and rectal bleeding, the degree of emaciation (defined as the reduction of subcutaneous adipose tissue, evaluated visually), and stool consistency. It was found that clinical symptoms tend to manifest differently in a sex dependent manner. For instance, rectal prolapse with bleeding was observed predominantly in male *Muc2*–/– mice. These findings highlight the importance of including both males and females in research samples when using this model.

**DOI:** 10.1134/S0022093025020115

**Keywords:** inflammatory bowel disease clinical symptoms, *Muc2*–/– mice, IBD model, prolapse, emaciation, muc-2-deficient mice

## INTRODUCTION

Inflammatory bowel diseases (IBD) are a prevalent group of chronic disorders [1], characterized by complex immune responses [2]. IBD is associated with a wide range of etiological factors, and current treatment methods primarily aim just to alleviate disease symptoms [3, 4].

Patients with IBD exhibit clinical symptoms, such as weight loss, fecal incontinence and urgency, abdominal pain, diarrhea, and rectal bleeding [5, 6]. However, evidence suggests that symptom severity and immune responses triggered by inflammation may vary between sexes. For instance, studies on the influence of sex on intestinal immune activity have shown that women exhibit a significantly stronger immune response to intestinal mucosal damage compared to men [7]. Notably, in most countries, IBD is more frequently diagnosed in men, as is the likelihood of developing colorectal cancer [8]. Moreover, men are diagnosed with IBD at an earlier age than women [9].

Various animal models are employed to study IBD [10], including induced models, such as dextran sulfate sodium (DSS)- and 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis, and others [11, 12], as well as transgenic models like *IL-10*–/–, *TGF-β*–/–, *Muc2*–/–, and others [13, 14]. In transgenic mice, the pathology develops more gradually, with physiological compensatory mechanisms miti-

gating inflammation, and the disease often enters prolonged remission. Thus, these models better replicate the clinical progression of IBD compared to induced models. One of the transgenic models widely used for IBD research over the past 20 years is *Mucin2* knockout (*Muc2–/–*) mice deficient in mucin 2, the primary mucosal glycoprotein. These mice develop mucosal insufficiency accompanied by inflammation [15]. *Muc2–/–* mice, originally created as a model for ulcerative colitis (a form of IBD), exhibit clinical symptoms similar to IBD patients, including histological and clinical features characteristic of the disease [15, 16]. These animals display diarrhea, rectal prolapse with bleeding, and weight loss [17, 18]. Clinical symptoms typically emerge by day 28 of life [19].

However, symptom manifestation can vary depending on the animal's age and individual variability [20]. For example, far not all knockout mice develop prolapse or stool abnormalities, even after six months of life [21]. Some cases exhibit reversible rectal prolapses, and rectal bleeding occurs not in all animals [16]. Over time, all IBD symptoms persist and deteriorate [21].

Thus, *Muc2–/–* mice display symptoms characteristic of IBD patients, albeit with minor age-related variations. Possible reasons for the variability in symptom onset include differences in researcher assessment and/or individual variability.

Nevertheless, most studies use *Muc2–/–* males aged 2–6 months, thereby complicating the evaluation of sex- and age-specific symptom manifestations. Meanwhile, there is evidence suggesting such differences do exist. For instance, in DSS-induced colitis, pathology severity was significantly higher in males compared to females, as evidenced by a greater weight loss and more pronounced clinical symptoms relative to controls [22]. Importantly, modern translational biomedical research emphasizes the need for sex-balanced study cohorts [23]. The paucity of data on pathology progression in female *Muc2–/–* mice hinders a comprehensive understanding of the pathogenesis in this model.

A clear understanding of the variability in IBD clinical symptoms is essential for grasping the disease's overall picture. For instance, symptom severity allows assessing disease progression and individual variability, providing insights into pathological mechanisms. The timing and degree of symptom manifestation may also reflect the efficacy (or lack thereof) of experimental treatments. Since clinical symptoms serve as visible markers of disease presence and severity in animals, changes in their timing or intensity can indicate alterations in disease progression.

The aim of this study was to evaluate the variability of IBD clinical symptoms in intact *Muc2–/–* mice depending on their sex and age.

## MATERIALS AND METHODS

*Animals.* The animals were housed under standard vivarium conditions in individually ventilated cages, with a 12/12-h light/dark cycle and ad libitum access to food and water. The vivarium maintained animal housing conditions in accordance with the State Standard (GOST) 33216-2014 [24]: temperature 20–24°C, air humidity 45–65%. The animals were fed PK-120-1 laboratory animal diet, prepared according to the GOST 34566-2019 "Complete feed for laboratory animals. Technical specifications."

*Muc2–/–* mice on the C57BL/6 genetic background were used in experiments. The animals were generated by A. Velcich at the Albert Einstein Cancer Center/Montefiore Medical Center [25] and obtained from the vivarium of the Research Institute of Neuroscience and Medicine, Siberian Branch of the Russian Academy of Sciences. Experimental cohorts were selected from the offspring of *Muc2–/–* and *Muc2+/+* mice derived by crossing heterozygous parents. Genotyping was performed on day 28 of life, after which the animals were separated. The littermates (siblings) were co-housed and later segregated by sex, with 1–5 animals per cage depending on litter size. A total of 24 *Muc2* animals were used in the study (Table 1).

*Clinical examination.* Clinical symptoms, including emaciation, rectal prolapse and bleeding, changes in body weight, and the presence of diarrhea, were assessed at a weekly basis in the morning. Body weight dynamics was evaluated using a SHINKO DENSHI scale (accuracy = 0.1 g). Each animal was thoroughly examined for signs of emaciation and rectal prolapse with bleeding. Notably, body weight gain does not always correlate with the degree of emaciation, so changes in body weight and emaciation severity were assessed as two individual parameters. Emaciation severity was evaluated using

**Table 1.** The number of animals (*n*) in the experimental groups (wt—wild type mice, het—heterozygous, muc—muc2 knockout mice)

| | Females | | | Males | | |
|---|---|---|---|---|---|---|
| Group name | $N_1$(f muc) | $N_2$(f wt) | $N_3$(f het) | $N_4$(m muc) | $N_5$(m wt) | $N_6$(m het) |
| Genotype | *Muc2–/–* | *Muc2+/+* | *Muc2+/–* | *Muc2–/–* | *Muc2+/+* | *Muc2+/–* |
| *n* | 3 | 4 | 4 | 3 | 4 | 6 |

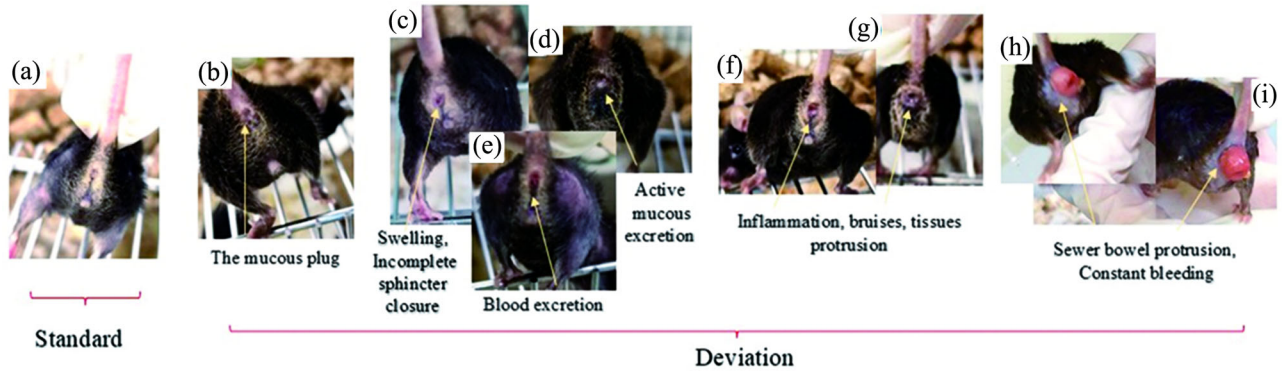


**Fig. 1.** An example of a prolapse scoring: (a) norm; (b) 1 point; (c–e) 2 points; (f, g) 3 points; (h, i) 5 points.

a scale proposed by The Icahn School of Medicine at Mount Sinai (USA) [35]. This visual scale reflects overall body condition and the degree of subcutaneous fat deposition. Clinical symptoms in *Muc2–/–* mice are commonly accepted to be assessed using the Disease Activity Index (DAI). The main DAI criteria include body weight loss, stool abnormalities (diarrhea), and altered general condition (fur quality, posture) [16, 26, 27]. DAI is used to assess disease severity not only in animals but also in patients with IBD [28].

Based on the published DAI protocols, we developed a more detailed version for assessing clinical symptoms of IBD, which included the following parameters: emaciation severity and rectal prolapse, presence of rectal bleeding, and diarrhea.

Below are the criteria for evaluating clinical symptoms (in score points):

Stool:

- No liquid stool in the cage—0;
- Liquid stool in the cage—1.

Emaciation:

- Norm—0;
- Mild—0.5;
- Moderate—1;
- Severe—2.

Rectal prolapse:

- Norm—0;
- Intensive mucus discharge (mucous plug)—1;
- Incomplete rectal prolapse (mucus with traces of blood or slight intestinal protrusion)—2;
- Complete rectal prolapse—3;
- Complete rectal prolapse with traces of blood—4;
- Complete rectal prolapse (severe) with active bleeding—5.

Examples of rectal prolapse and bleeding severity are shown in Fig. 1.

*Statistical data* processing. Statistical analysis was carried out using GraphPad Prism 8. The nonparametric Mann–Whitney test was used for pairwise comparisons. All clinical symptom scores are presented as median values per animal. Body weight data are shown as the average body weight of all animals in a group on the day of measurement. Data on plots represent mean values ± standard deviation ($M \pm SD$).

## RESULTS

By week 13 of life, most *Muc2–/–* females demonstrated the signs of emaciation, which persisted or, in some cases, exacerbated by week 23 (Figs. 2, 3).

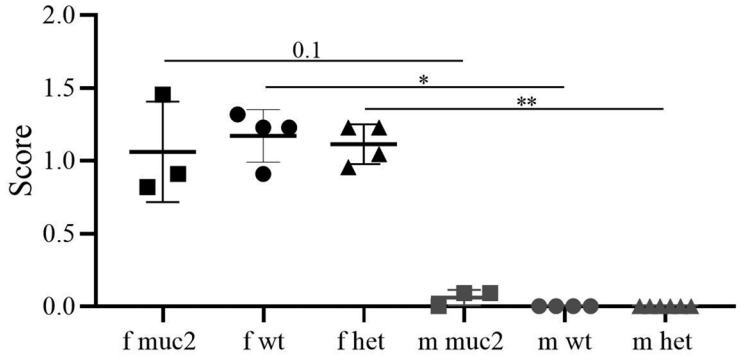


**Fig. 2.** Emaciation degree (in score points) in mice, as assessed in the period from week 13 to week 23 of life and presented as the average emaciation score of the animal over the measurement period. * $p < 0.05$; $N_1$(f muc) = 3, $N_2$(f wt) = 4, $N_3$(f wt) = 4, $N_4$(m muc) = 3, $N_5$(m wt) = 4, $N_6$(m wt) = 6; Mann–Whitney test.

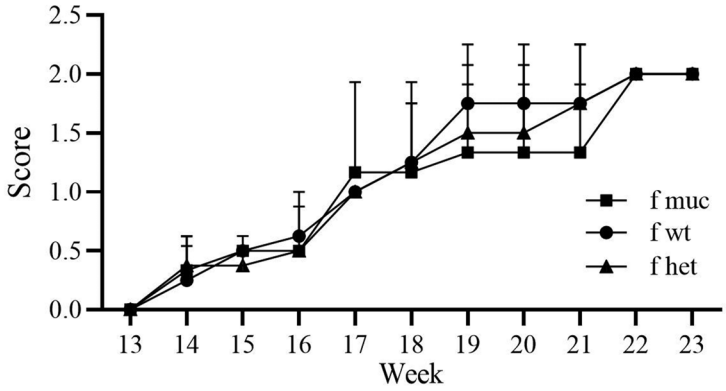


**Fig. 3.** Emaciation dynamics in female mice, as assessed in all animals of the same group. Data are presented as the median value and interquartile range; *ordinate*: emaciation score points. $N_1$(f mac) = 3, $N_2$(f wt) = 4, $N_3$(f het) = 4.

The degree of emaciation in females did not depend on genotype. Similarly, no signs of emaciation were observed in male *Muc2–/–* mice during the study period. Males of both wild-type and heterozygous genotypes, as well as *Muc2–/–*, showed no signs of emaciation throughout the experiment. At the same time, a statistically significant difference in the degree of emaciation was observed between wild-type females and males ($p < 0.05$), as well as between heterozygous females and males ($p < 0.01$). The degree of emaciation in *Muc2–/–* females was slightly higher compared to *Muc2–/–* males, though this difference was only a trend ($p = 0.1$) (Fig. 2).

Thus, as for emaciation, the obtained data confidently indicate that the differences in this trait are associated with sex rather than genotype: emaciation is significantly higher in all females compared to all males, and this difference persists when comparing females to males across any of the genotypes.

When analyzing emaciation dynamics (Fig. 3) in females of different genotypes, it can be observed that, regardless of genotype, all females exhibit signs of moderate emaciation by week 17 of life, which progresses over time. This suggests that emaciation develops similarly in females of any genotype, and in some cases, the degree of emaciation in control animals may exceed that in *Muc2–/–* females. It is worth noting that the later onset of severe emaciation in knockout females is due to the significant individual variability in IBD symptoms, which, with a small number of animals, may produce such a pattern due to random factors. Ultimately, by the end of the observation period, the degree of emaciation in females of all genotypes reaches its maximum value. Thus, it appears that emaciation parameters in wild-type and knockout females may overlap, making this indicator unsuitable for distinguishing between them.

Over the period of measuring clinical IBD symptoms, after the third week of measurements (week 15

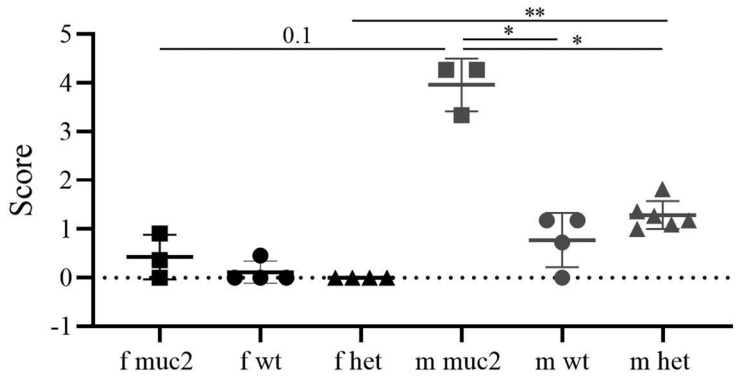


**Fig. 4.** The degree of rectal prolapse and bleeding, as assessed in score points in the period from week 13 to week 23 of life and presented as the average score of rectal prolapse with or without bleeding during the measurement period. * $p < 0.05$, ** $p < 0.01$; $N_1$(f mac) = 3; $N_2$(f wt) = 4; $N_3$(f et) = 4; $N_4$(m) = 3; $N_5$(mvt) = 4; $N_6$(m hat) = 6; Mann–Whitney test.

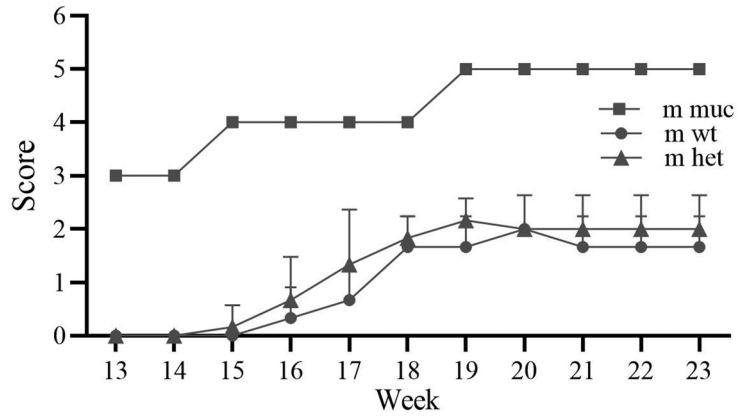


**Fig. 5.** Rectal prolapse and bleeding dynamics in male mice (median values), expressed in score points to assess prolapse manifestations. $N_4$(m muc) = 3; $N_5$(m wt) = 4; $N_6$(m het) = 6.

of life), one of the *Muc2–/–* males reached the criteria for a humane experimental endpoint (severe bleeding and extreme intestinal prolapse) and was euthanized. By week 13 of life, most *Muc2–/–* females exhibited almost no signs of rectal prolapse and absolutely no signs of rectal bleeding. The signs of rectal prolapse in *Muc2–/–* females emerged in some cases by week 23 of life (data not shown). At the same time, no signs of rectal prolapse were observed in wild-type or heterozygous females (Fig. 4).

In almost all *Muc2–/–* males, the signs of rectal prolapse in the form of mucous plugs (initial stage) were observed by week 13 of life, and in some cases, rectal prolapse was already evident by the third month (Fig. 1). By week 23 of life, most *Muc2–/–* males developed rectal prolapse with traces of blood, and in some cases, severe rectal prolapse with constant bleeding was detected. Meanwhile, in most wild-type males, the signs of rectal prolapse were observed closer to the end of the measurement period and did not reach the extreme stage, whereas in many heterozygous males, rectal prolapse developed more prominently compared to wild-type males. Thus, the severity of rectal prolapse and bleeding in *Muc2–/–* males was significantly higher than in wild-type ($p < 0.05$) and heterozygous ($p < 0.05$) males. At the same time, the degree of rectal prolapse was slightly higher (as a trend, $p = 0.1$) in *Muc2–/–* males compared to *Muc2–/–* females and significantly higher in heterozygous males compared to heterozygous females ($p < 0.01$) (Fig. 4).

Thus, in contrast to emaciation, it can be confidently stated that the degree of prolapse manifestations is associated with genotype: it is significantly higher in mucin-deficient males compared to males of other genotypes.

Initial and moderate stages of rectal prolapse

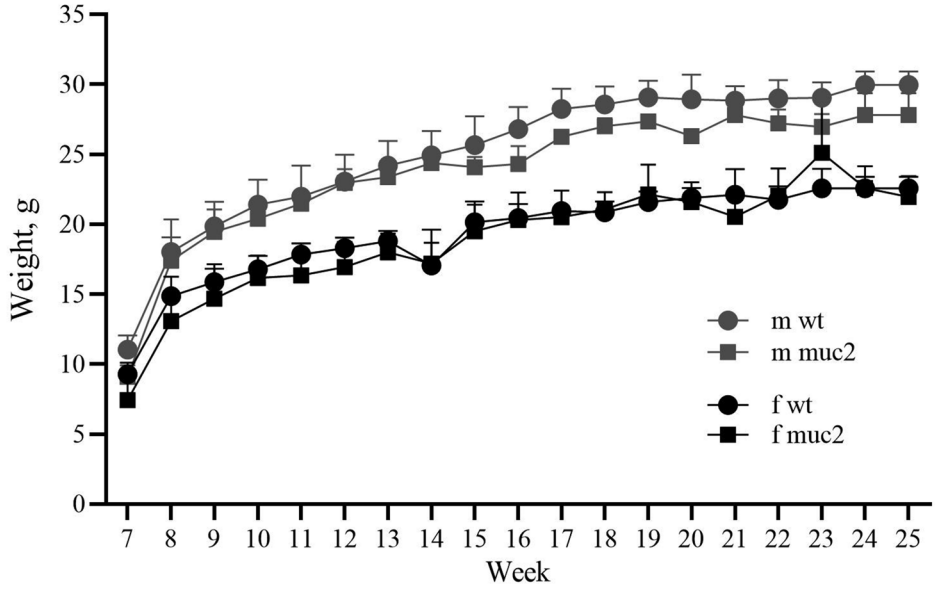


**Fig. 6.** Weight gain dynamics in mice over the period from week 7 to week 25 of life. Data are presented as the average weight of all animals of a certain sex and genotype at the point studied. $N_1$(f muc) = 3; $N_2$(f wt) = 4; $N_4$(m muc) = 3; $N_5$(m wt) = 4.

(intestinal prolapse and/or initial prolapse with abundant mucus secretion) were observed in *Muc2–/–* males as early as week 13 of life (Fig. 5). At the same time, in wild-type and heterozygous males, initial-stage prolapse (mucous discharge and mucous plugs, as well as initial prolapse with abundant mucus secretion), appeared only by the beginning of weeks 15–16. Subsequently, the degree of prolapse manifestation in heterozygotes and wild-type mice reached the initial level characteristic of *Muc2–/–* mice and did not progress further.

It is noteworthy that intestinal prolapse is irreversible, whereas mucous discharge, mucous plugs, and detectable initial-stage rectal prolapse may be transient in nature.

The dynamics of body weight gain in the mice during the measurement period correspond to data from other studies [16, 29]. As expected, all females had lower body weight than males, regardless of genotype (Fig. 6), which mirrors the pattern observed in the C57Bl/6 strain, the genetic background of the model used in this study [30]. It is evident that *Muc2–/–* females showed a slight lag in body weight gain during the first weeks of measurement compared to wild-type females, but this difference diminished over time. However, the opposite pattern was observed in males. From week 14 onward, *Muc2–/–* males began to lag in body weight gain compared to wild-type males, which aligns with literature data [16]. Nevertheless, the difference in weight between knockout and wild-type males was less pronounced compared to other studies [16, 17, 22], where in some cases, body weight loss could reach 30–40% [22].

Here, we also assessed stool abnormalities in the mice. Signs of diarrhea were observed, but its occurrence did not correlate with prolapse development, nor did the presence of prolapse guarantee diarrhea. When calculating the frequency of stool abnormalities, we found the signs of diarrhea in 8 out of 11 females, with only three females developing first-stage prolapse (mucous discharge, swelling) by the middle or end of the measurement period. In contrast, diarrhea was observed in only 4 out of 13 males, of which only two exhibited severe rectal prolapse. Another important fact is that signs of diarrhea were not persistent throughout the experiment. Stool abnormalities could appear, disappear, and reappear over time. Thus, signs of diarrhea and altered stool were more frequently observed in females than in males (data not shown).

We also noted signs of alopecia in the animals. Temporary or permanent alopecia, manifested as hair thinning on the head and body, was observed in 5 females. Signs of alopecia were also present in males. In our experiment, we observed complete

lack of whiskers and hairless snouts in 3 out of 4 wild-type males. In the experimental mice, alopecia was also not persistent, while being more frequently observed in females.

## DISCUSSION

Clinical symptoms of IBD are a major indicator of IBD manifestation in both humans and animal models. These symptoms allow for the assessment of disease severity as they reflect the organism's integral response. In *Muc2–/–* mice, clinical symptoms are used not only to detect the disease and evaluate the severity of its progression but also to verify the efficacy of therapeutic interventions.

However, the variability of these symptoms complicates the process, making the study of baseline symptom variability fundamentally important. This work is among the first to evaluate sex-specific differences in IBD symptom manifestation between male and female *Muc2–/–* mice (previous work by Fijneman RJA et al. [31] demonstrated that male and female *Muc2–/–* mice have different frequency and dynamics of tumor emergence). We managed to detect a reduction in body weight in *Muc2–/–* males compared to wild-type males, though the degree of reduction was less pronounced than reported in other studies. In females, we noted a slight lag in weight gain among *Muc2–/–* individuals during the initial weeks of measurement, after which their weight matched that of wild-type females. In *Muc2–/–* males, we also observed severe rectal prolapse accompanied by persistent bleeding. Rectal prolapse was not exclusive to knockout males but also occurred in heterozygous and wild-type males [16]. Conversely, signs of emaciation in females were evident from the first weeks of measurement. Females of each genotype exhibited significant emaciation symptoms that progressed over time, while rarely displaying signs of rectal prolapse. Throughout the measurement period, no *Muc2–/–* female exhibited complete intestinal prolapse; only early stages were observed, occasionally accompanied by bloody discharges. Heterozygous and wild-type females either showed no signs of rectal prolapse or only the presence of a mucous plug (the first indicator of potential intestinal prolapse).

It is well known that IBD symptoms are not solely caused by inflammatory processes but are also mediated by changes in gut microbiota composition. Given that mice of different genotypes were co-housed throughout the experiment, it is possible that some of the observed symptoms were more influenced by shared microbiota than by inflammatory processes associated with mucin 2 deficiency [32].

Thus, in co-housed siblings, the dynamics of IBD clinical symptoms in *Muc2–/–* mice does not allow for unambiguous discrimination between homozygotes, heterozygotes, and wild-type individuals. Our study revealed a varying diagnostic significance of IBD symptoms: emaciation and diarrhea do not reliably differentiate wild-type from knockout mice, whereas prolapse, especially when severe, allows for such a differentiation.

A second important finding is the sex-specific significance of symptoms. For instance, prolapse in males clearly distinguishes knockout males from wild-type animals, whereas in females, it does not.

When using this model in studies suggesting the assessment of the disease activity index (DAI) score in response to therapy, sex-specific and age-related dynamics of clinical symptoms must be considered. Such studies must include both males and females, as co-housing of mucin-deficient mice with siblings of other genotypes helps differentiate microbiota-mediated symptoms from those caused by the lack of mucin. Notably, modern biomedical research standards necessitate the inclusion of both sexes, which echoes with our findings [33].

In this study, siblings were co-housed and separated only by sex. Previous studies have shown that when mice from different litters (non-siblings) were separated and housed by both sex and genotype, more severe IBD symptoms and consequences, including tumors, ensued [34]. One of the possible explanations of this phenomenon is a chronic social stress caused by intragroup aggression when unrelated adult mice are co-housed. Chronic stress may serve a trigger, exacerbating IBD symptoms in *Muc2–/–* mice compared to their non-stressed counterparts. We believe that in the case of co-housed siblings, the presence and apparent severity of clinical symptoms in wild-type and heterozygous mice are mediated by microbiota, shared with knockout animals.

Therefore, when interpreting the outcomes from studies involving *Muc2–/–* mice, housing and grouping formats must be considered. To mark out

the clinical consequences of mucin deficiency, studies should use mixed-sex cohorts with co-housed siblings of different genotypes.

## AUTHORS' CONTRIBUTION

Conceptualization and experimental design (V.S.P., K.S.M.), data collection and processing (K.S.M.), writing and editing the manuscript (V.S.P., K.S.M.).

## FUNDING

This work was supported by the Russian Foundation for Basic Research (project no. 19-75-30007; https://rscf.ru/project/19-75-30007). No additional grants to conduct or supervise this particular research were obtained.

## ETHICS APPROVAL

Animal-related experiments were carried out in compliance with the NIH Guidelines for the care and use of laboratory animals (http://oacu.od.nih.gov/regs/index.htm), as well as the State Standard of the Russian Federation (GOST) 33216-2014 [24]. Experimental protocols and all animal manipulations were approved by the Bioethics Committee of Moscow State University (no. 3.6-h. of 19/05/2024 and no. 183-a of 12/08/2024).

## CONFLICT OF INTEREST

The authors of this work declare that they have no conflict of interest.

## REFERENCES

1. Knyazev OV, Shkurko TV, Kagramanova AV, Veselov AV, Nikonov EL (2020) Epidemiology of inflammatory bowel disease. State of the problem (review). Russ J Evidence-Based Gastroenterol 9: 66–73. https://doi.org/10.17116/dokgastro2020902166
2. Chen C, Xu J, Han T, Chen G, Yu K, Du C, Shen W, Sun Y, Zeng X (2024) Microencapsulation as a Protective Strategy for Sialylated Immunoglobulin G: Efficacy in Alleviating Symptoms of Dextran Sulfate Sodium-Induced Colitis in Mice and Potential Mechanisms. J Agric Food Chem 72: 4074–4088. https://doi.org/10.1021/ACS.JAFC.3C07733/SUPPL_FILE/JF3C07733_SI_001.PDF
3. Liu F, Smith AD, Solano-Aguilar G, Wang TTY, Pham Q, Beshah E, Tang Q, Urban JF, Xue C, Li RW (2020) Mechanistic insights into the attenuation of intestinal inflammation and modulation of the gut microbiome by krill oil using in vitro and in vivo models. Microbiome 8: 1–21. https://doi.org/10.1186/S40168-020-00843-8/FIGURES/9
4. Fantini MC, Guadagni I (2021) From inflammation to colitis-associated colorectal cancer in inflammatory bowel disease: Pathogenesis and impact of current therapies. Digestive and Liver Disease 53: 558–565. https://doi.org/10.1016/J.DLD.2021.01.012
5. Colombel JF, Shin A, Gibson PR (2019) AGA Clinical Practice Update on Functional Gastrointestinal Symptoms in Patients With Inflammatory Bowel Disease: Expert Review. Clin Gastroenterol Hepatol 17: 380–390. e1. https://doi.org/10.1016/J.CGH.2018.08.001
6. Yu YR, Rodriguez JR (2017) Clinical presentation of Crohn's, ulcerative colitis, and indeterminate colitis: Symptoms, extraintestinal manifestations, and disease phenotypes. Semin Pediatr Surg 26: 349–355. https://doi.org/10.1053/J.SEMPEDSURG.2017.10.003
7. Sankaran-Walters S, Macal M, Grishina I, Nagy L, Goulart L, Coolidge K, Li J, Fenton A, Williams T, Miller MK, Flamm J, Prindiville T, George M, Dandekar S (2013) Sex differences matter in the gut: effect on mucosal immune activation and inflammation. Biol Sex Differ 4: 10. https://doi.org/10.1186/2042-6410-4-10
8. Knyazev OV, Shkurko TV, Fadeeva NA, Bakulin IG, Bordin DS (2017) Epidemiology of chronic inflammatory bowel diseases. Yesterday, today, tomorrow. Exp Clin Gastroenterol 139: 4–12. (In Russ).
9. Malaty HM, Hou JK, Thirumurthi S (2010) Epidemiology of inflammatory bowel disease among an indigent multi-ethnic population in the United States. Clin Exp Gastroenterol 3: 165–170. https://doi.org/10.2147/CEG.S14586
10. Singh Jaggi A, Kaur Randhawa P, Singh K, Singh N (2014) A Review on Chemical-Induced Inflammatory Bowel Disease Models in Rodents. Korean J Physiol Pharmacol 18: 279–288. https://doi.org/10.4196/kjpp.2014.18.4.279
11. Kim JJ, Shajib MS, Manocha MM, Khan WI (2012) Investigating Intestinal Inflammation in DSS-

induced Model of IBD. J Vis Exp 60: 3678. https://doi.org/10.3791/3678
12. Kawada M, Arihiro A, Mizoguchi E (2007) Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. World J Gastroenterol 13: 5581. https://doi.org/10.3748/WJG.V13.I42.5581
13. Mizoguchi A (2012) Animal Models of Inflammatory Bowel Disease. Prog Mol Biol Transl Sci 105: 263–320. https://doi.org/10.1016/B978-0-12-394596-9.00009-3
14. Bhan AK, Mizoguchi E, Smith RN, Mizoguchi A (1999) Colitis in transgenic and knockout animals as models of human inflammatory bowel disease. Immunol Rev 169: 195–207. https://doi.org/10.1111/J.1600-065X.1999.TB01316.X
15. Wenzel UA, Magnusson MK, Rydström A, Jonstrand C, Hengst J, Johansson MEV, Velcich A, Öhman L, Strid H, Sjövall H, Hansson GC, Wick MJ (2014) Spontaneous Colitis in Muc2-Deficient Mice Reflects Clinical and Cellular Features of Active Ulcerative Colitis. PLoS One 9: e100217. https://doi.org/10.1371/JOURNAL.PONE.0100217
16. Van der Sluis M, De Koning BAE, De Bruijn ACJM, Velcich A, Meijerink JPP, Van Goudoever JB, Büller HA, Dekker J, Van Seuningen I, Renes IB, Einerhand AWC (2006) Muc2-Deficient Mice Spontaneously Develop Colitis, Indicating That MUC2 Is Critical for Colonic Protection. Gastroenterology 131: 117–129. https://doi.org/10.1053/j.gastro.2006.04.020
17. Borisova MA, Achasova KM, Morozova KN, Andreyeva EN, Litvinova EA, Ogienko AA, Morozova MV, Berkaeva MB, Kiseleva E, Kozhevnikova EN (2020) Mucin-2 knockout is a model of intercellular junction defects, mitochondrial damage and ATP depletion in the intestinal epithelium. Scient Rep 10: 1–17. https://doi.org/10.1038/s41598-020-78141-4
18. Estaki M, Morck DW, Ghosh S, Quin C, Pither J, Barnett JA, Gill SK, Gibson DL (2020) Physical Activity Shapes the Intestinal Microbiome and Immunity of Healthy Mice but Has No Protective Effects against Colitis in MUC2 –/– Mice. mSystems 5(5): e00515. https://doi.org/10.1128/MSYSTEMS.00515-20/SUPPL_FILE/MSYSTEMS.00515-20-SF007.EPS
19. Paassen NB Van, van der Sluis M, Bouma J, Korteland-Van Male AM, Lu P, van Seuningen IV, Boehm G, van Goudoever JB, Renes IB (2011) Colitis development during the suckling-weaning transition in mucin muc2-deficient mice. Am J Physiol Gastrointest Liver Physiol 301: 667–678. https://doi.org/10.1152/AJPGI.00199.2010/SUPPL_FILE/FIGURE4-REV.PDF
20. Milutinovich KS, Popov VS (2024) Variability of histological features in the IBD model of mucin 2-deficient mice. CHEMBIOSEASONS 2024: 185. (In Russ).
21. Morampudi V, Dalwadi U, Bhinder G, Sham HP, Gill SK, Chan J, Bergstrom KSB, Huang T, Ma C, Jacobson K, Gibson DL, Vallance BA (2016) The goblet cell-derived mediator RELM-β drives spontaneous colitis in Muc2-deficient mice by promoting commensal microbial dysbiosis. Mucosal Immunol 9: 1218–1233. https://doi.org/10.1038/mi.2015.140
22. Gao Yu, Postovalova EA, Dobrynina MT, Makarova OV (2016) Sex differences in morphological changes and immune disorders in experimental acute ulcerative colitis. Clin Exp Morphol 1: 37–43. (In Russ).
23. Karp NA, Katial R, Thacker K (2020) Sex in studies: The first level of personalisation. Physiol News 118: 32–35. https://doi.org/10.36866/PN.118.32
24. GOST 33216-2014. Guidelines for the care and maintenance of laboratory animals. Rules for the maintenance and care of laboratory rodents and rabbits. Accessed 4 Dec 2024. (In Russ). https://internet-law.ru/gosts/gost/62388/. Accessed 4 Dec 2024
25. Velcich A, Yang WC, Heyer J, Fragale A, Nicholas C, Viani S, Kucherlapati R, Lipkin M, Yang K, Augenlicht L (2002) Colorectal cancer in mice genetically deficient in the mucin Muc2. Science 2959(5560): 1726–1729. https://doi.org/10.1126/SCIENCE.1069094/SUPPL_FILE/1069094S7_THUMB.GIF
26. Zhou Y, Ji G, Yang X, Chen Z, Zhou L (2023) Behavioral abnormalities in C57BL/6 mice with chronic ulcerative colitis induced by DSS. BMC Gastroenterol 23: 1–10. https://doi.org/10.1186/S12876-023-02718-2/FIGURES/6
27. Murthy SNS, Cooper HS, Shim H, Shah RS, Ibrahim SA, Sedergran DJ (1993) Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin. Dig Dis Sci 38: 1722–1734. https://doi.org/10.1007/BF01303184
28. Lan D, Niu J, Miao J, Dong X, Wang H, Yang G, Wang K, Miao Y (2016) Expression of guanylate cyclase-C, guanylin and uroguanylin is downregulated proportionally to the ulcerative

colitis disease activity index. Scient Rep 2016 6: 1–6. https://doi.org/10.1038/srep25034
29. Park YH, Kim N, Shim YK, Choi YJ, Nam RH, Choi YJ, Ham MH, Suh JH, Lee SM, Lee CM, Yoon H, Lee HS, Lee DH (2015) Adequate Dextran Sodium Sulfate-induced Colitis Model in Mice and Effective Outcome Measurement Method. J Cancer Prev 20: 260–267. https://doi.org/10.15430/JCP.2015.20.4.260
30. Body Weight Info—B6 000664 The Jackson Laboratory. https://www.jax.org/jax-mice-and-services/strain-data-sheet-pages/body-weight-chart-000664. Accessed 6 Jan 2025
31. Fijneman RJA, Peham JR, van de Wiel MA, Meijer GA, Matise I, Velcich A, Cormier RT (2008) Expression of Pla2g2a prevents carcinogenesis in Muc2-deficient mice. Cancer Sci 99: 2113–2119. https://doi.org/10.1111/J.1349-7006.2008.00924.X
32. Bets VD, Achasova KM, Borisova MA, Kozhevnikova EN, Litvinova EA (2022) Role of Mucin 2 Glycoprotein and L-fucose in Interaction of Immunity and Microbiome within the Experimental Model of Inflammatory Bowel Disease. Biochemistry (Moscow) 87: 301–318. https://doi.org/10.1134/S0006297922040010/FIGURES/5
33. Phillips B, Haschler TN, Karp NA (2023) Statistical simulations show that scientists need not increase overall sample size by default when including both sexes in in vivo studies. PLoS Biol 21(6): e3002129. https://doi.org/10.1371/journal.pbio.300212934
34. Wu M, Wu Y, Li J, Bao Y, Guo Y, Yang W (2018) The Dynamic Changes of Gut Microbiota in Muc2 Deficient Mice. Int J Mol Sci 19: 2809. https://doi.org/10.3390/ijms19092809
35. Body condition scoring for mice. The Icahn School of Medicine at Mount Sinai (USA). https://icahn.mssm.edu/files/ISMMS/Assets/Research/CCMS/BodyConditionScoringForMice.pdf

*Translated by A. Polyanovsky*