Original Article

Effects of Remdesivir (GS-5734) on Anti-Müllerian Hormone Level and Ovarian Tissues Morphology in Experimental Female Rats

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Abstract

Background: Remdesivir (GS-5734), an antiviral drug, recommended for the management of pandemic COVID-19 patients. This emergency medicine has not been fully investigated for its possible adverse effects on female reproductive capacity. **Objective:** To investigate the effects of remdesivir on the reproductive function through biochemical and morphological evaluation of ovaries, and to assess the conception outcomes and pups physical development in albino rat model study. **Materials and Methods:** A total of 24 adult female rats were equally divided into two groups. Experimental group was given a dose of 5 mg/kg of remdesivir intra-peritoneally for 10 consecutive days at intervals of 24 h. One day after treatment, half of rats (IIA) were weighted and sacrificed. The remaining rats (IIB) were housed with male. Following parturition, the weight of mothers and pups were measured, and the indices of physical development of nursing pups were recorded. Later, mothers were scarified on day 60 from last exposure. During the experiment, serum levels of anti-Mullerian hormone (AMH) was measured and the ovaries were subjected to morphologic and morphometric assessments. All data were analyzed using SPSS version 2.0 for statistically significant difference. **Results:** The mean concentration of AMH for the control group and the experimental groups (IIA) and (IIB) was 4.276 ± 1.582, 1.818 ± 1.554, and 3.339 ± 1.219 ng/mL, respectively. The IIA group had the lowest AMH level. There was slight recovery in the AMH level in experimental group IIB. The mean count of atretic follicles and corpus lutea was significantly different in experimental group IIA, with *P*-values of 0.041 and 0.033, respectively. The indices of physical development of nursing pups were significantly low ($P \leq 0.05$). **Conclusion:** Remdesivir could produce an intermittent reproductive disruption shortly after administration manifested by transient decline in ovarian reserve. Therefore, more attention should be paid to when this medicine is prescribed for women, especially for reproductive-aged women seeking for near-future pregnancy.

Keywords: Anti-mullerian hormone, ovary, rats, remdesivir

Introduction

The emergence of COVID-19, a novel coronavirus disease, has become a major public health crisis. Such a pandemic highlights an urgent need for therapeutics.[1]

Remdesivir (GS-5734; Gilead Sciences Inc., Foster City, California, USA) is Food and Drug Administration approved antiviral drug and is recommended for the management of COVID-19 patients by the World Health Organization.[2] The active metabolite of remdesivir is a "nucleoside analog" which acts as a competitive inhibitor of the viral reverse transcription in COVID-19 patients.[3] Such a mechanism of action reduces viral load by inhibiting its replication. The drug is administered intravenously with an initial loading dose of 200 mg on day 1, followed by a 100 mg maintenance dose given from day 2 through day 10.[4]

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It is well-known that successful reproduction is fundamental to the continuation of all livings. The assessment of safety of medications is determined through examining the integrity of reproductive tissues because any defect in the female reproductive capacity may indicate abnormalities in those tissues. Such potential insult could be investigated through hormonal and morphological analysis of the reproductive organs.[5]

Anti-Müllerian hormone (AMH) serves as a highly effective ovarian reserve biomarker in predicting reproductive function and indicating any iatrogenic ovarian follicle damage.[6] It is a valuable serum marker for evaluating ovarian reserve post-medication.[7] This glycoprotein is part of the transforming growth factor-β (TGF-β) family and is primarily secreted by granulosa cells of the antral follicles, with higher serum levels indicating a greater ovarian follicle count, thus AMH can predicts the woman future fertility lifespan.[8]

The potential effects of this nucleoside analog drug on reproductive hormones and ovarian tissues have not been fully investigated.[9] Therefore, this emergency medicine has caused patients considerable worry not only in terms of the COVID-19 virus itself but also of this drug used in management protocols.[10]

Since limited current data are available on the reproductive safety of remdesivir, this study aimed to explore the potential impact of remdesivir on female reproductive function through biochemical and morphological evaluation of rat ovaries, and to assess the conception outcomes and offspring physical development.

Materials and Methods

This experimental study was conducted at the veterinary medicine animal house of Mosul University. A total of 24 female rats (4-5 months of age) weighing between 200 and 250 g were used. These animals were maintained in cages in pathogen-free settings that included free access to water and food with virtual humidity (30%–50%), accurate lighting (12/12 h light–dark cycle), and controlled temperature (18–22°C). Before the experiment began, rats were given two weeks to adjust to the new environments. The weight of the animals was estimated using an electronic balance. The experimental protocol was authorized by the Ethical Committee of the Faculty of Veterinary Medicine, University of Mosul (Ref: UM.VET.2022.051).

Drug used

Remdesivir was provided by Eva Pharma, a generic drug maker has received a license from Gilead Sciences Ireland to make remdesivir in a dose of 100 mg/20 mL vial.[2]

Experimental design

The rats were randomly divided into two groups, with 12 rats in each group. Group I, the control group, given daily oral distilled water for 10 consecutive days. Group II, the experimental group, animals were intraperitoneally injected with remdesivir for 10 consecutive days (10 mg/kg body weight on day one, followed by 5 mg/kg body weight daily). One day after completing the treatment, half of the rats (IIA) were weighed and sacrificed. The remaining rats (IIB) underwent vaginal smears to observe their estrous cycle, with subsequent housing with male rodents, and continued vaginal smearing to check for pregnancy.[11]

After parturition, the average number of new birth pups was recorded and the weight of mothers and pups was measured at birth, 7th, 14th, and 21st days. The indices of physical development and growth of nursing pups were recorded. The nursing pups were monitored daily until they were weaned on day 22, for the appearance of fur, lower incisor teeth eruption, the date of eye openings which occurred when pups both eyes were at least partially opened, and the appearance of ear lobes.[12] At the end of the experiment and after weaning of pups, the mother rats were sacrificed on day 70th.

Samples collection

The rats were anesthetized and blood samples were obtained immediately from the retro-orbital vessels by microcapillaries, and then animals were sacrificed by cervical dislocation. The serum samples were obtained by centrifuging the whole blood at 3000 rpm for 15 min and the supernatants were transferred into the Eppendorf tubes for the biochemical analysis. Subsequently, a midline abdominal incision was performed and the abdominal cavity was opened up and the ovaries were excised and cleansed in saline, weighed, and immediately fixed in 10% neutral buffered formalin for 24 h. The relative weights were obtained by dividing the reproductive organ weight by the body weight and multiplying it by 100, expressed as grams per 100 g of body weight. The tissue was processed through dehydration using a series of ethanol concentrations, followed by clarification, and finally embedding in paraffin blocks.

Biochemical analysis

The concentrations of anti-Müllerian hormone (AMH), serum fertility hormones (luteinizing hormone (LH), and follicle stimulating hormone (FSH)) were measured using an "enzyme-linked immunosorbent assay" according to the Siiteri.[13] All hormone concentrations were evaluated using a kit bought from ELK Biotechnology company (Wuhan), following the instructions provided by the manufacturer.

Histopathological evaluation

By using a microtome, 5 µm sections were performed (8 sections per animal/group). To avoid recounting of follicles, every 10th section was stained with hematoxylin

and eosin (HE) and scanned (Aperio Image Scope 9.0). Histological changes including; ovarian fibrosis, atrophy, vascular congestion, edema, and chronic inflammatory cell infiltration were also reported when present and graded in to negative, mild, moderate, and severe accordingly. Ovarian atrophy is scored according to the degree of reduced numbers of follicles and corpora lutea.

Morphometric analysis

The ovarian follicles were counted independently by two pathologists according to a method of the previously described study.[14]

The criteria for recognizing follicles relied on the specific types of surrounding epithelial cells. For instance, primordial follicles refer to oocytes that were enveloped by a layer of cuboid granulosa cells. In contrast, primary follicles were oocytes surrounded by diverse layers of cuboidal granulosa cells.[15]

Statistical analyses

The obtained results were analyzed using Statistical Package for the Social Sciences (SPSS, IBM® Inc., Chicago, Illinois, USA) (version 20) and expressed as mean ± SD of different groups. The significance was calculated by analysis of variance (ANOVA) and Student's "*t*" test. The *P*-value equal or less than 0.05 was considered statistically significant.

Results

Body weight and ovary weight

There were progressive body weight gains of animals throughout the experiment. However, no significant correlation was detected between both groups (P = 0.063). The ovarian weight was statistically low in the experimental group (IIA) [Table 1].

Biochemical analysis

A significant difference was observed in the levels of AMH, FSH, and LH between the control and experimental groups, with *P*-values of 0.001, 0.012, and 0.031, respectively. Specifically, AMH level was significantly lower in the experimental group (IIA) compared to both control and experimental group (IIB), whereas FSH level was markedly elevated in both experimental groups in comparison to the control. LH concentration was high in the experimental group (IIA) versus the control and experimental group (IIB). The data are summarized in Table 2.

Histopathological evaluation

The histopathological changes in rats' ovaries of both groups are illustrated in Table 3. The control group revealed normal histology and architecture of the ovary with numerous growing follicles in different stages with multiple corpora lutea [Figure 1].

In the experimental subgroup IIA, moderate ovarian atrophy and fibrosis were observed, scattered among several follicles at various stages of maturation [Figure 2]. Evidence of tissue degeneration and necrosis is present, accompanied by signs of an inflammatory response, including vascular congestion, edema, and heavy chronic inflammatory cell infiltration [Figure 3].

The section of ovaries in the experimental subgroup IIB showed an improvement in the ovarian tissues by mild ovarian atrophy and fibrosis, along with a reduction in inflammation, vascular congestion, and edema [Figure 4].

Table 1: The combined weight of the total body and ovaries in adult female rats

Variables	CTR no. (12)	EXP 10th days no. (6)	EXP 70th days no. (6)
Total body weight (g)	250.90 ± 5.33 a	253.53 ± 6.34 a	254.53 ± 6.44 a
Absolute ovarian weight (mg)	41.30 ± 2.62 a	37.45 ± 2.41 b	40.10 ± 2.47 a
Relative ovarian weight (mg/100 g b.w)	16.22 ± 1.00 a	14.65 ± 1.18 b	15.98 ± 1.00 a

no: number of animals in each group, CTR: control group, EXP: experimental group

Data are expressed as means ± SEM

Different letters indicate statistically significant differences *P*-value ≤ 0.05

Table 2: Effect of remdesivir on serum hormones level of FSH, LH, and AMH in (ng/mL) of adult female rats

Hormones	CTR no. (12)	EXP 10th days no. (6)	EXP 70th days no. (6)
FSH (ng/mL)	3.059 ± 0.59b	9.282 ± 0.174a	6.134 ± 0.992a
LH (ng/mL)	0.354 ± 0.546b	0.674 ± 0.488a	0.291 ± 0.196b
AMH (ng/mL)	4.276 ± 1.582a	1.818 ± 1.554b	3.339 ± 1.219a

no: number of animals in each group, CTR: control group, EXP: experimental group

Data are expressed as means ± SEM

Different letters indicate statistically significant differences *P*-value ≤ 0.05

Table 3: The histopathological changes of ovaries in the control and experimental groups of adult female rats

Variables	CTR no. (12)	EXP 10th days no. (6)	EXP 70th days no. (6)
Ovarian atrophy	–	++	+
Vascular congestion	–	++	+
Chronic inflammatory cell infiltration	–	+++	+
Interstitial Fibrosis	–	++	+
Edema	–	++	+

no: number of animals in each group, CTR: control group, EXP: experimental group, –: absent, +: Mild, ++: Moderate, +++: Severe

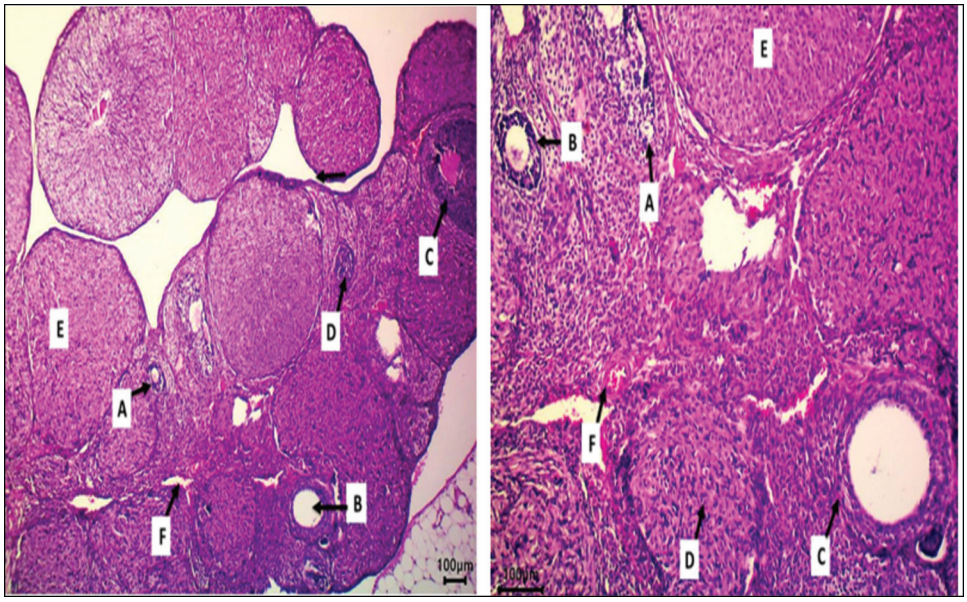

Figure 1: Photomicrographs of ovarian rat of control group showing the normal ovarian architecture representing primary (A), secondary (B), tertiary (C), atretic follicles (D), corpora luteum (E), and blood vessels (F). HE stain, left 40× and right 100×

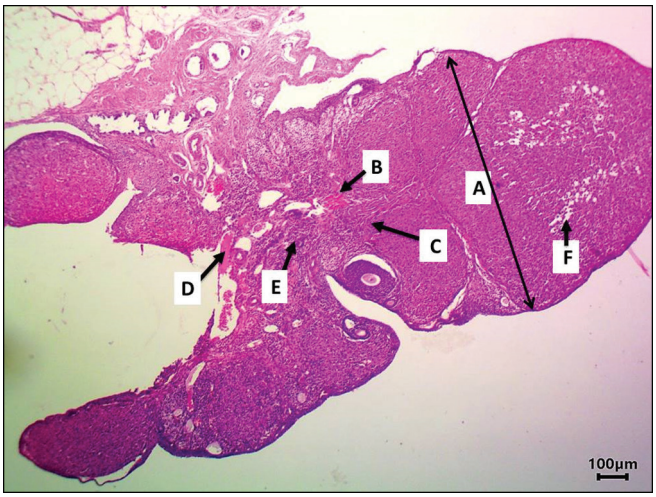

Figure 2: Photomicrograph of ovarian rat of experimental group (IIA) showing ovarian atrophy (A), vascular congestion (B), fibrosis (C), edema (D), atretic follicles (E), and degenerative cells in the corpora luteum (F). HE stain, 40×

Morphometric analysis

The ovaries of both groups revealed the presence of ovarian follicles in variable stages of differentiation. Both cortex and medulla were identified. All follicles were present in the cortex of the ovary.

In the experimental group (IIA), a significant reduction in the number and size of primordial follicles, primary follicles, and secondary follicles was observed; all were associated with *P*-values < 0.05 [Table 4 and Figure 5]. It was also observed that the atretic follicles were significantly increased as compared to control and experimental group (IIB) [$P = 0.041$; Table 4 and Figure 6]. The corpora luteal count was decreased in the experimental group (IIA) in comparisons to the control and experimental group (IIB) [$P = 0.033$; Table 4], some of them revealed degenerated cells with deeply stained esinophilic cytoplasm and eccentric pyknotic nuclei [Figure 2].

In the experimental group (IIB), there was a recovery in the number of follicles [Table 4 and Figure 7].

Analysis of physical development of nursing pups

The experimental dams revealed a significantly lower number of pups compared to the control dams, with a *P*-value of 0.04. The control and experimental pups

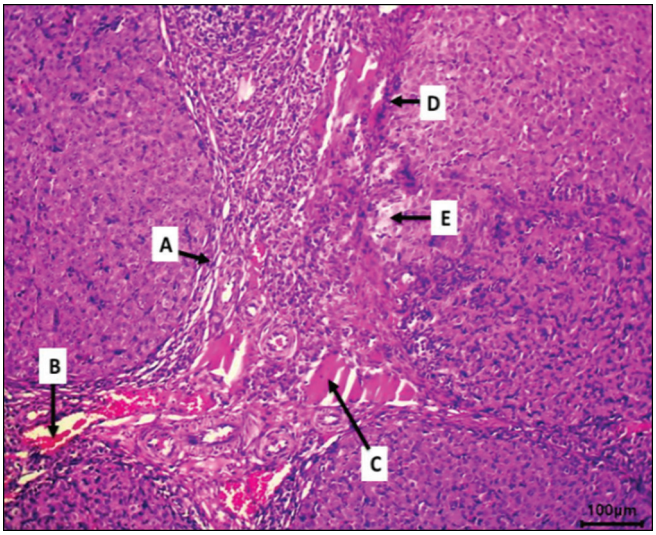

Figure 3: Photomicrograph of ovarian rat of experimental group (IIA) showing the fibrosis (A), vascular congestion (B), interstitial edema (C), chronic inflammatory cells infiltration (D), and necrosis (E). HE stain, 100×

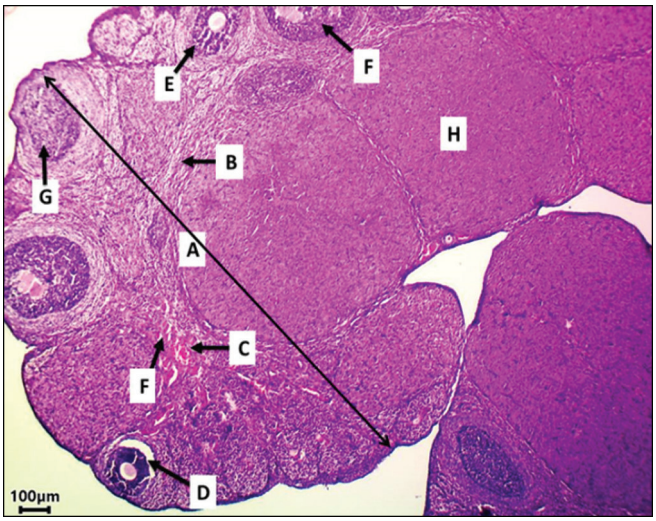

Figure 4: Photomicrograph of ovarian rat of experimental group (IIB) showing mild ovarian atrophy (A), fibrosis (B), vascular congestion (C), interstitial edema (D), secondary follicle (E), tertiary follicles (F), atretic follicle (G) and corpora luteum (H). HE stain, 40×

significantly differed in the days of the appearance of the ear lobe, fur, lower incisor eruption, and eye-opening [$P \leq 0.05$; Table 5]. Additionally, when assessing the rate of body weight gain, a significant difference was found between both groups. Starting from PND14 and PND21 [Table 6], the control pups exhibited significantly higher body weight compared to the experimental pups, whereas the control and lactating mothers revealed no significant weight difference.

Discussion

Remdesivir, the first licensed antiviral drug for the treatment of COVID-19, has uncertain impact on female reproductive capacity due to limited clinical trial experience data.[15]

In this research, the dosing regimen of remdesivir was designed to closely mimic the therapeutic doses used in clinical settings for humans.[16] The administration of remdesivir did not have an impact on the body weight of female rats throughout the experiment. However, morphological examination revealed a significant loss of ovarian weight in the experimental group versus the control group, parallel with findings from a previous study.[17]

It is widely recognized that the key aspect of female reproductive function is the cyclical activity, and any defect in this process can negatively affects the reproductive potential.[18] The assessment of female reproductive function is commonly done through the ovarian reserve test, a valuable and widely utilized method. However, the most reliable parameter for determining ovarian reserve is the histological examination and subsequent counting of ovarian follicles.[19] Unfortunately, this approach is not practical and unfeasible for humans, even in research purposes. Therefore, a rat model was performed to investigate the effects of remdesivir on folliculogenesis, owing to their relatively short cycle length, which typically averages around 4 days.[6]

During the study, ovarian reserve parameters, including follicles counting and AMH level, were reduced on the

Table 4: A comparison of the average ovarian follicular count between control and experimental groups of adult female rats

Variables	CTR no. (12)	EXP 10th days no. (6)	EXP 70th days no. (6)	*P*-value
No. of primordial follicles	3.75±0.75 a	1.25±0.34 b	2.50±0.64 a	0.043
No. of primary follicles	2.5±0.28 a	1.15±0.28 b	1.75±0.25 a	0.028
No. of secondary follicles	2.5±0.28 a	1.25±0.25 b	2.5±0.25 a	0.016
No. of tertiary follicles	1.5±0.28 a	0.5±0.28 b	0.75±0.25 b	0.046
No. of atretic follicles	6.75±0.47 a	15.0±1.87 b	9.0±1.68 a	0.041
No. of corpora lutea	15.75±1.43 a	10.0±1.18 b	12.25±2.25 a	0.033

no: number of animals in each group, No: Number, CTR: control group, EXP: experimental group

Different letters indicate significant differences *P*-value ≤ 0.05

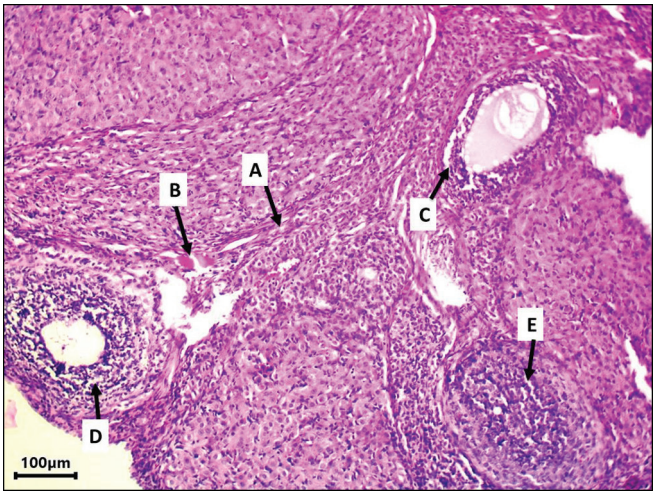

Figure 5: Photomicrograph of ovarian rat of experimental group (IIA) showing fibrosis (A), edema (B), primary (C) and secondary follicle with degeneration and necrosis (D), many atretic follicles (E). HE stain, 100×

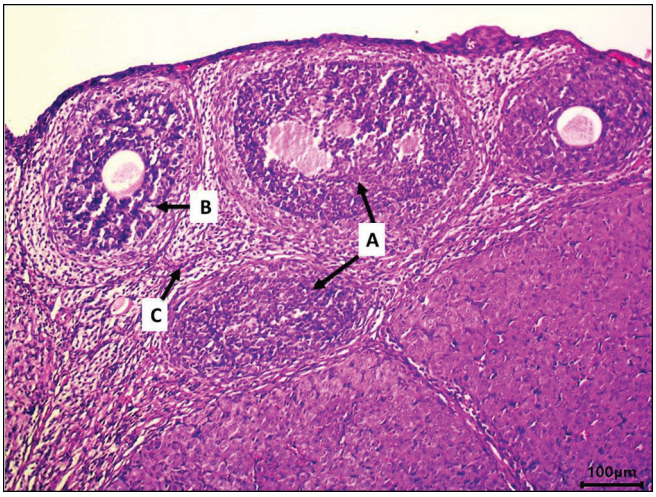

Figure 6: Photomicrograph of ovarian rat of experimental group (IIA) showing many atretic follicles (A) and secondary follicle with degeneration (B) surrounding by fibrosis (C). HE stain, 100×

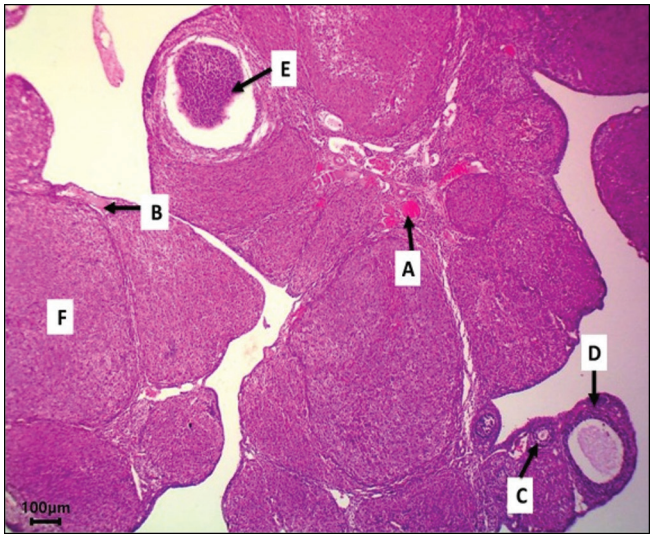

Figure 7: Photomicrograph of rat ovary of experimental group (IIB) showing vascular congestion (A), primary (B), secondary follicle (C), atretic follicles (D&E), and corpora luteum (F). HE stain, 100×

10th day post administration of remdesivir compared to the control group. ADditionally, histological examination revealed a reduction in folliculogenesis, with several follicles ending up as atretic follicles. Moreover, a decline in corpora lutea, particularly observed on the 10th day from medication application. These results are consistent with those of Knauff *et al*.[20]

Normal ovarian function relies on the interactive and integrated feedback mechanisms of the "hypothalamic-pituitary-ovarian" (HPO) axis.[21] However, when there is reduced quality and quantity of ovarian follicles, it disrupts this negative feedback mechanism, resulting in an elevation in FSH and LH levels.

In the study, decreased folliculogenesis was reflected in the high FSH and LH levels, indicating a poor ovarian reserve.[22] We hypothesized that remdesivir may have a deleterious effect on ovarian reserve and endocrine function. Further, we supposed that the impact of remdesivir on ovaries should be taken into account when using AMH as an assessment tool for ovarian reserve.

In parallel with our results, a prior study examined the reproductive toxicity of remdesivir in a rat model reported noticeable effects on ovarian function, including severe reduction in corpora lutea.[23] Further, Awodele and his colleagues,[24] demonstrated the effect of active anti-retroviral drugs on the reproduction of rats, they reported a decline in follicular development and reproductive hormones concentrations in female rodents that received active anti-retroviral therapy compared to controls. Unfortunately, there is a lack of clinical studies investigating the impact of remdesivir on human reproduction for comparisons.

Ovarian fibrosis is primarily caused by ovarian injury triggered by different factors such as heavy chronic inflammatory cell infiltrations. This inflammatory response can lead to the release of chemoattractants, which stimulate fibroblasts to produce collagen, ended with tissue remodeling.[25] This pathological finding was reported in our study through increased inter-follicular connective tissue fibers. Further, ovarian atrophy was reported, through the reduced numbers of follicles and corpora lutea.[26] It was assumed that the atrophy observed in the ovaries was a chemically induced by the effect of remdesivir, particularly targeting primordial and primary follicles. This was manifested by the prominence of atretic follicles and the low corpora leutea.[26] Fortunately, most of these deleterious effects decreased after 60 days from drug discontinuation. Therefore, we assumed that the injurious effects of remdesivir on the ovary are transient and it will decline with time following the sensation of the

Table 5: A comparison of the effect of remdesivir on the average rat pups growth factors (days) between the control and experimental groups

G. parameters groups	Appearance of ear lobe	Appearance of fur	Lower incisor teeth eruption	Appearance of eye-opening
Control pups	2.33 ± 0.21	5.66 ± 0.33	8.50 ± 0.34	12.00 ± 0.25
Mother treated pups	3.17 ± 0.3*	7.66 ± 0.33*	11.17 ± 0.47*	13.66 ± 0.42*

G: growth

*The value (mean ± standard error) differed significantly between the groups at the level $P \leq 0.05$

Table 6: A comparison of the effect of remdesivir on the average weight of nursing rat pups (gm) between the control and experimental groups

Days groups	At parturition	At seventh PND	At fourteenth PND	At twenty-one PND
Control	6.70 ± 0.3	8.02 ± 0.22	9.77 ± 0.19	16.45 ± 1.06
Mother treated pups	6.49 ± 0.1 NS	8.29 ± 0.32 NS	8.90 ± 0.26*	14.99 ± 0.77*

PND: post-natal day

*The value (mean ± standard error) differed significantly between the groups at the level $P \leq 0.05$

medicine. However, those recorded transient side effects of remdesivir on the ovary should be taken into consideration, especially for reproductive-aged women planning near-future pregnancy.[27]

In the study, the recommended human dose (RHD) of remdesivir did not affect female rat conception. However, a reduction in the number of newborn pups was reported, this was comparable with previous rat study.[17] We believe that the decline in corpora lutea function played a significant role in the reduction of litter size.

In experimental nursing rats, reduction in the body weight and growth parameters were noticed. Those findings were in parallel with a systematic review of remdesivir therapy for pregnancy,[27] but inconsistent with other study.[17] Some would suppose that the lowered growth parameters of nursing pups might be attributed to the prenatal effect of remdesivir; however, the exact explanation is not known. This opinion was supported by evidence from a study revealed that remdesivir active metabolite could pass through the placenta.[28] Moreover, we supposed that remdesivir might cause a reduction in the pregnant rodents appetite and intestinal absorption. Further investigation of such issue should be conducted in future studies.

Our study clarified that remdesivir can induce pathologic changes in ovarian stroma leading to disruption in folliculogenesis and endocrine function. However, it is important to note that most of these changes were reversible after drug discontinuation. Future studies will need to investigate whether or not there is evidence of such adverse effects after administration of remdesivir. ADditionally, future studies should examine the health of offspring born to females who received remdesivir shortly before pregnancy.

Limitations to our study include the lack of use of different dosage regimens of remdesivir to determine the dose-related toxicity. ADditionally, we did not conduct a complimentary assessment of the oxidative stress and antioxidant markers, which might have provided valuable insights.

On the other hand, the study has notable strengths including the use of a rat model allowed us to study the broader impact of remdesivir beyond its direct effects on follicles and reproductive hormones. The attention given to the stromal environment, which contributes to the health of follicles, provided a comprehensive understanding of the subject.

Conclusion

Remdesivir could produce an intermittent reproductive disruption shortly after administration manifested by transient decline in ovarian reserve. Therefore, more attention should be paid to when this medicine is prescribed for females, especially for reproductive-aged women seeking for near-future pregnancy.

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Conflicts of interest

There are no conflicts of interest.

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