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Original Article

Systemic Effect of Human Follicular Fluid from Endometriotic and Healthy Subjects on Female Mice

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ABSTRACT

Aim/Background: Throughout oogenesis and folliculogenesis, the follicular fluid's composition alters physiologically to fit the needs of particular microenvironmental demands. This study's main goal was to compare the effect of follicular fluid collected from endometriotic and non-endometriotic patients on systemic body functioning of female mice. **Materials and Methods:** Both healthy and endometriotic participants' follicular fluid was collected and pooled separately. Female Swiss albino mice were injected with 1 and 2 mL/kg/day endometrial fluid in the intraperitoneal region and monitored for 21 days. Change in body weight, hormonal profile, glucose profile and hematological profile was monitored and recorded on regular basis. **Results:** On day 21, the blood glucose level increased from 100.2±0.96 mg/dL to 138.4±3.32 mg/dL. Endometriotic follicular fluid had a dose-dependently decreased serum estradiol from 28.80±0.37 to 27.00±1.0 ng/mL and progesterone form 24.17±0.7 to 1.72±0.21 pg/mL and a rise in testosterone levels from normal 3.95±0.81 nmol/mL to 9.4±0.92. It has elevated serum LH levels to approximately three times normal levels. In contrast, the serum FSH level decreased from 19.40±0.74 mIU/mL to 2.5±0.22. As a result, the LH to FSH ratio increased from 0.18±0.01 to 3.9±0.19. There was a dose-dependent rise in serum insulin level significantly (p<0.001) from normal 0.74±0.02 IU/mL to 1.63±0.05 and 2.09±0.1 respectively. In a similar manner, HOMA-IR also showed increase in insulin resistance from normal 0.17 to 0.57±0.02 and 0.71±0.04. HOMA-Beta normal level was 8.17±0.3 increased to 10.05±0.4 beta cell dysfunction. Nevertheless, QUICKI were both dosages dependently decreased insulin sensitivity from 1.31±0.07 dose dependently to 0.9±0.01and 0.83±0.02. **Conclusion:** Female mice treated with endometriotic follicular fluid of endometriosis patients displayed pancreatic abnormality. It has been concluded that endometriotic patients' follicular fluid not only has a localized effect but also contains elements that enter the systemic circulation, have negative effects, and may be connected to significant clinical symptoms of endometriotic condition.

Keywords: Biological fluid, Cystic fluid, Endometriotic, Follicular fluid, Ovarian fluid, Systemic effects.

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INTRODUCTION

Endometriosis refers to the presence of endometrial glands and stromal lesions outside the uterus. These lesions can manifest as deep infiltrating disease, ovarian cysts, superficial implants, or peritoneal lesions. The exact cause of endometriosis remains unknown, but there are several theories explaining the formation of endometriotic lesions. One potential mechanism is retrograde menstruation, where the endometriotic lining is expelled into the pelvic cavity through the fallopian tubes. Along with possible circulation through blood or lymph, this retrograde flow may lead to the implantation of endometriotic cells in ectopic sites. The persistence of these lesions in the pelvic cavity might be influenced by additional factors such as inflammation, hormones, or the immune system.[1]

Endometriosis can result from Müllerian remnants or trans differentiation of circulating blood cells. It is an inflammatory condition where endometrium-like tissue is found outside the uterus. Symptoms include dysmenorrhea, infertility, pelvic pain, and fatigue. Endometriosis poses socioeconomic consequences similar to chronic diseases like diabetes mellitus. Guidelines vary due to treatment complexity. About 10-15% of reproductive-age women, and 70% of those with chronic pelvic pain, have endometriosis.[2] Unfortunately, many women with endometriosis suffer unnecessarily and have a lower quality of life due to

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delayed diagnosis. Patients between 18 and 45 years old typically experience a delay of 6.7 years.[3] Early consultation, diagnosis, and treatment can alleviate pain, halt disease progression, and preserve fertility, as symptoms often start in adolescence.[4] However, obstacles like high costs of testing and therapy in adolescents, along with confounding signs such as cyclic and acyclic pain, hinder early diagnosis. Therefore, a non-invasive diagnostic tool for endometriosis could enable earlier.[5] Current proposed markers, including immunologic, serum, and genetic markers, lack the necessary sensitivity and specificity for use as screening tests. Most researchers agree that retrograde menstrual flow through the fallopian tubes may contribute to endometriosis development. Endometriotic tissue-derived progenitor cells can implant in various locations, causing chronic inflammation, pelvic adhesions, and infertility.[6] Genetic, endocrine, anatomical, and environmental factors influence endometriosis susceptibility.[7] Clinical evidence suggests that endometriosis worsens over time, leading to increased pain and subfertility.[8] The severity of endometriosis correlates with reduced fertility, with spontaneous conception possible for about half of women with mild or moderate cases.[9] To diagnose Leutinized Unruptured Follicle (LUF) syndrome, intact follicles on repeated ultrasound scans are necessary as hormone tests are inconclusive. Women with endometriosis have a higher risk of developing LUF syndrome compared to those without the condition. Research indicates that the use of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), commonly prescribed for dysmenorrhea, increases the risk of LUF syndrome by blocking follicle rupture through the inhibition of prostaglandin production in the ovaries.[10] Abnormal uterine contractions caused by endometriosis, specifically uterine tubal dysperistalsis, may result in infertility due to impaired migration of zygotes and embryos.[11]

The composition of follicular fluid varies depending on localized genitourinary pathophysiology. It not only has local physiological effects but may also have systemic effects. Therefore, this study aims to evaluate the systemic effects of follicular fluid collected from patients diagnosed with endometriosis and non-endometriosis on female mice.

MATERIALS AND METHODS

Selection of subjects

Patients with endometriosis are selected through a physical examination (palpation) that identifies uterine or adnexal tenderness, a retroverted uterus, nodulating uterosacral ligaments, and pelvic masses. It was then followed by ultrasonography, a non-invasive technique. The absence of these clinical characteristics indicated a normal, healthy population. The male companions of these normal, healthy women were identified as the cause of their IVF treatment. Case severity was not considered in the selection of patients.

Collection of follicular fluid

Regularly menstruating, healthy women and those with endometriosis who were having IVF at Indira IVF Centre, Dehradun, India provided follicular fluid. To achieve regulated ovarian hyperstimulation, all women underwent a protracted IVF treatment involving a GnRH agonist and recombinant FSH combination. About 34 to 36 hr following the delivery of human Chorionic Gonadotropin (hCG) (10,000 IU). During transvaginal ultrasound-guided oocyte aspiration, fluid was collected from three follicles of >12 mm in diameter from each patient and pooled. Further fluid from 10 endometriotic patients and 10 non-endometriotic individuals were separately pooled. The samples of follicular fluid used for examination were macroscopically clean and free of flushing medium contamination. To remove cellular debris, the samples were centrifuged at 10,000 g for 30 min at 4°C. Syringe-filtered (0.22 m) supernatants were then kept at 3-4°C pending further usage.[12,13] This work has been approved by the University Research Ethics Committee of DIT University, Dehradun and Indira IVF Centre, Dehradun. Written informed consents were obtained from all participants.

Animals and groups

Animal studies were carried out after the approval by Institutional Animals Ethics Committee. All animal study protocols were as per the Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines. Adult female 30 mice aged 60 days, were used in this experiment. No chemical or medicinal treatments were given to the animals. The mice were randomly split into five groups of six each. The first group (the control) consisted of mice that were given only a standard laboratory meal and water along with normal saline 5 mL/kg i.p. Group II and III mice received normal patient's follicular fluid injected 1 mL/kg and 2 mL/kg i.p respectively. Whereas, Group IV and V mice received endometric patient's follicular fluid injected 1 mL/kg and 2 mL/kg i.p respectively.

Blood sampling method and sample handling

The animals were fasted overnight and euthanized with overdose of ketamine on last day of the experiment after withdrawing 2 mL of blood in vacutainer for biochemical investigation. For histological examination kidney, liver, lung, pancreas, spleen, heart and ovary were isolated and stored in 10% neutral buffered formalin.[14]

Biochemical analysis

Pathology lab centre in Dehradun performed the biochemical analysis. The biochemical constituents' concentrations were determined in accordance with the pathology laboratory's guidelines.[15]

Biochemical Parameters

The levels of blood glucose were measured utilising digital glucometer. By employing ERBA Fertikit an immuno enzymometric test was used to quantify the serum levels of LH and FSH. Insulin was estimated using diagnostic kit from Span Diagnostic Ltd. India. A 96-well plate ELISA microplate reader (MultiskanTM GO, Thermo Fischer scientific) was used to measure serum levels of estradiol, progesterone, and testosterone using the GenXbio kit.[16]

Estimation of HOMA-IR, HOMA-Beta and QUICKI

HOMA-IR was calculated using the formula fasting insulin (lU/mL) X fasting glucose (mmol /L)/22.5 (1) and QUICKI as 1 /[log fasting insulin (lU/ mL)+log glucose (mg / dL)]. HOMA 1-%B=(20X FPI)/(FPG-3.5).[17]

Hematological estimation methods

Following the advice of Dacie and Lewis (2006), blood was collected for hepatological investigation in a tube containing Dipotassium Ethylene Diamine Tetra Acetate (EDTA) to prevent clotting. Total blood cells, red blood cell count, platelet counts, hemoglobin concentration, mean corpuscular volume, and mean corpuscular hemoglobin concentration are examples of Hematological Metrics (MCHC) using a hematological Analyzer (GI-HA3000). Moreover, blood samples were obtained for biochemical analysis and spun for 20 min at 25000 rpm without the use of any anticoagulants in a centrifuge tube. Glass tubes were used to segregate clear serum samples before they were put through several biochemical tests.[18]

Histopathological examinations

Using the usual procedures, isolated organs were processed, embedded in paraffin, sectioned, deparaffinized, and rehydrated. Examining the microscopic changes allowed researchers to gauge the full impact of the follicular fluid-induced modifications. Hematoxylin and eosin (H and E) was used to stain tissue slices, which were then viewed under a microscope.[19]

Statistical analysis

Means and standard deviations of the data are reported (Mean±SEM). Student t-test was used to determine if differences were significant, and a value of $p<0.05$ was deemed to indicate statistical significance.

RESULTS

Sample characteristics

The Shapiro-Wilk test confirms that the data is normally distributed therefore we analysed the data by student *t*-test followed by One-Way ANOVA.

Effect on hematological profile

Treatment of mice for 21 days with normal follicular fluid resulted in significant ($p<0.001$) increase in RBC (red blood cells), platelet, hemoglobin, hematocrit, MHC (mean corpuscular hemoglobin), and P-LCR (Platelet-large cell ratio). Whereas significant decrease in MCV (Mean corpuscular volume) ($p<0.001$) and RDW-CV (red cell distribution width-Coefficient of Variation). Similarly, treatment with endometriotic follicular fluid significantly increased MCV ($p<0.001$), RDW-CV ($p<0.001$), RDW-SD (red cell distribution width-standard deviation) ($p<0.001$), MPV (mean platelet volume) ($p<0.001$), PCT (plateletcrit) ($p<0.01$), and P-LCR ($p<0.001$). But multiple factors such as haemoglobin ($p<0.05$), haematocrit ($p<0.05$), MHC ($p<0.001$), MCHC (mean corpuscular hemoglobin concentration) ($p<0.001$), and PCT ($p<0.01$) were significantly reduced (Table 1).

Change in body weight

Figure 1 shows the results of a 21-day chronic endometriosis and normal follicular fluid treatment on female mice. On days 14, a normal follicular fluid therapy of 2 mL/kg resulted insignificantly increase in body weight from 25.98±1.25 g to 28.2±0.66 at 1 mL/kg and 28.76±0.4 at 2 mL/kg. Similarly, on days 21 there was no significant change in body weight with normal follicular fluid. On days 14 and 21, female mice body weight decreases in a highly significant ($p<0.001$) manner at 2 mL/kg. When compared to day 1 (27.6±0.67 g), a very substantial ($p<0.01$) decrease at a dose of 2 mL/kg was seen on days 14 (25.9±0.68 g) and 21 (25.92±0.65 g).

Blood glucose level

During treatment with parenteral endometriosis follicular fluid, the blood glucose level in female mice increased highly considerably ($p<0.001$) at both 1 and 2 mL/kg. It was discovered to be dose and treatment time dependent. On day 21, the blood glucose level increased by 2 mL/kg, from 100.2±0.96 mg/dL to 126.2±2.81 and 138.4±3.32 mg/dL on day 14 and 21 respectively. Similar results were obtained by treatment with 1 mL/kg from 100.2±0.86 to 125.4±2.04 and 142.2±2.88 mg/dL on day 14 and 21 respectively. The symptoms of hyperglycemia were absent with normal follicular fluid treatment, as seen in Figure 2.

Serum estradiol, progesterone and testosterone

Female mice treated with normal follicular fluid at 1 and 2 mL/kg did not alter any level of hormones significantly. However, treatment with endometriotic follicular fluid had a dose-dependent decrease ($p<0.001$) in serum estradiol from 28.80±0.37 in normal to 27.00±1.0 ng/mL at 2 mL/kg and progesterone 24.17±0.70 normal levels to 1.94±0.11 and 1.72±0.21 pg/mL with 1 and 2 mL/kg endometriotic fluid respectively ($p<0.001$) and a rise in testosterone levels from normal 3.95±0.81 nmol/mL which was highly significantly ($p<0.001$) to 8.8±0.37 and 9.4±0.92 at 1 and 2 mL/kg endometriotic fluid (Figure 3).

Table 1: Changes in hematological profile of mice due to follicular fluid parenteral administration.

	Normal control	Normal follicular fluid 1 mL/kg	Normal follicular fluid 2 mL/kg	Endo follicular fluid 1 mL/kg	Endo follicular fluid 2 mL/kg
RBC	7.780±0.2905	9.620±0.07348***	10.18±0.09695***	8.140±0.1122	8.120±0.05831
PLT	989.0±13.64	1048±13.74***	1075±7.772***	1006±1.327	993.4±3.789
HGB	13.12±0.1772	13.84±0.09274**	14.46±0.1435***	14.18±0.0800*	14.02±0.1655*
MCV	43.26±0.2502	40.50±0.1844***	39.84±0.1435***	42.56±0.1691***	40.50±0.2236***
HCT	40.16±0.1208	40.80±0.2550*	41.28±0.1158***	40.70±0.3000*	41.14±0.0887*
MHC	14.52±0.1772	15.20±0.09487**	15.52±0.1772**	13.84±0.1435	13.24±0.09274***
MCHC	33.52±0.1594	34.10±0.1304*	35.36±0.2731***	33.90±0.2280***	35.46±0.2040***
RDW-CV	16.40±0.2811	15.34±0.1887**	16.28±0.1319	18.04±0.1288***	19.18±0.1114***
RDW-SD	29.70±0.1225	30.30±0.20008	31.00±0.2739**	32.74±0.1786***	34.90±0.3449***
PDW	10.54±0.1631	10.74±0.1122	11.24±0.2293*	11.30±0.2000**	11.60±0.2915**
MPV	10.60±0.1871	10.90±0.1871	11.00±0.08367*	16.20±0.1225***	16.90±0.1000***
PCT	0.6160±0.02249	0.6180±0.03308	0.6520±0.003742	0.6520±0.008**	0.6300±0.0221**
P-LCR	2.000±0.1581	2.600±0.1703*	2.620±0.08602**	3.400±0.1871***	3.500±0.2236***

Data expressed in Mean±SEM n=6/group, endo=endometriosis.

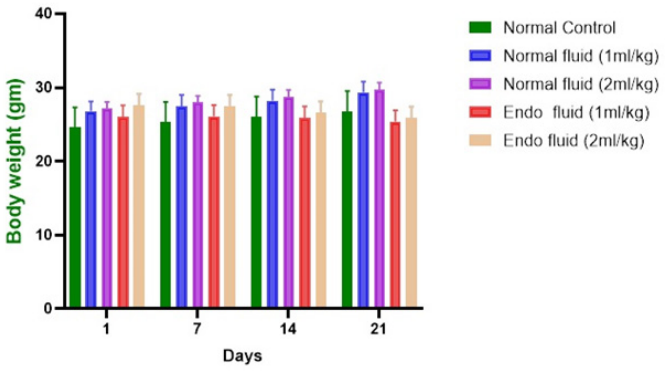

Figure 1: Influence of Follicular Fluid Parenteral Treatment on Change in Body Weight: shows the results of a 21-day chronic endometriosis and normal follicular fluid treatment on female mice.

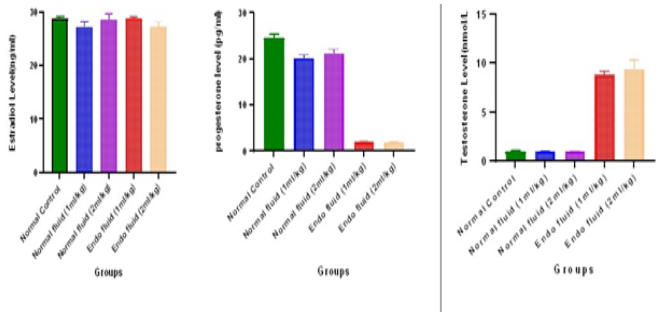

Figure 3: Effect of Follicular Fluid Parenteral Treatment on Serum Estradiol, Progesterone and Testosterone Levels. Female mice treated with endometriosis follicular fluid had a dose-dependent decrease in serum estradiol and progesterone levels and a rise in testosterone levels, which was highly significant (p<0.001).

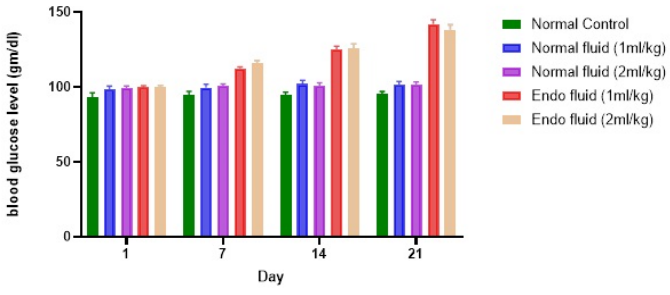

Figure 2: Effect of Follicular Fluid Parenteral Treatment on Blood Glucose Level: Treatment with parenteral endometriosis follicular fluid, the blood glucose level in female mice increased highly considerably (p<0.001).

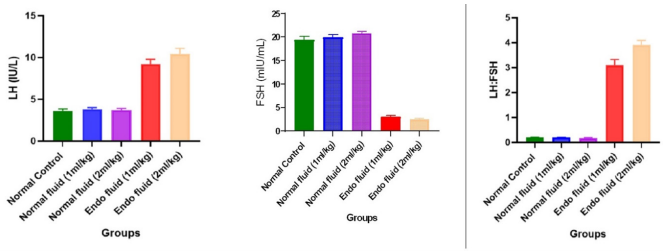

Figure 4: Effect of Follicular Fluid Parenteral Treatment on Change in Serum LH, FSH and LH: FSH Ratio. Female mice were treated with intravenous endometriosis follicular fluid at doses of 1 and 2 mL/kg, and both treatments significantly (p<0.001) elevated serum LH levels to approximately three times normal levels.

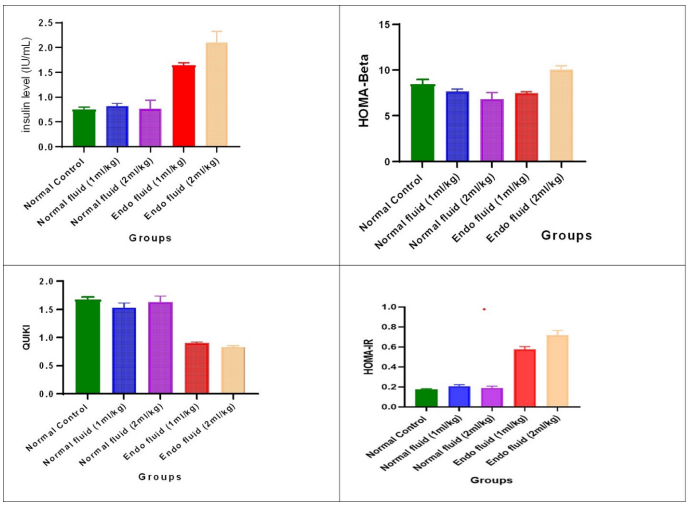

Figure 5: Effect of Follicular Fluid Parenteral Treatment on Insulin Sensitivity and Resistance; There was a dose-dependent rise in serum insulin level that was observed that was extremely significant (p<0.001). In a similar vein, HOMA-IR and HOMA-Beta also showed substantial significance (p<0.001). Nevertheless, QUICKI were both dosage dependently and highly substantially (p<0.001) decreased.

Serum LH, FSH and ratio

Female mice were treated with intraperitoneal endometriotic follicular fluid at doses of 1 and 2 mL/kg, and both treatments significantly (p<0.001) elevated serum LH levels from normal 3.60±0.24 IU/L to approximately three times normal levels (Figure 4). In contrast, the serum FSH level decreased from 19.40±0.74 mIU/mL decreased to 3.06±0.32 and 2.5±0.22 at the doses of 1 mL/kg and 2 mL/kg of endometriotic follicular fluid (p<0.001). As a result, the LH to FSH ratio increased from 0.18±0.01 to 3.08±0.23 and 3.9±0.19 in a highly significant (p<0.001) manner respectively (Figure 4).

Insulin sensitivity and resistance

In an effort to comprehend the impact of endometriotic follicular fluid on insulin level, insulin sensitivity and insulin resistance on female mice, 1 and 2 mL/kg were parenterally supplied for 21 days (Figure 5). There was a dose-dependent rise in serum insulin level was observed significantly (p<0.001) from normal 0.74±0.02 IU/mL to 1.63±0.05 and 2.09±0.1 respectively. In a similar manner, HOMA-IR (Homeostatic Model Assessment of

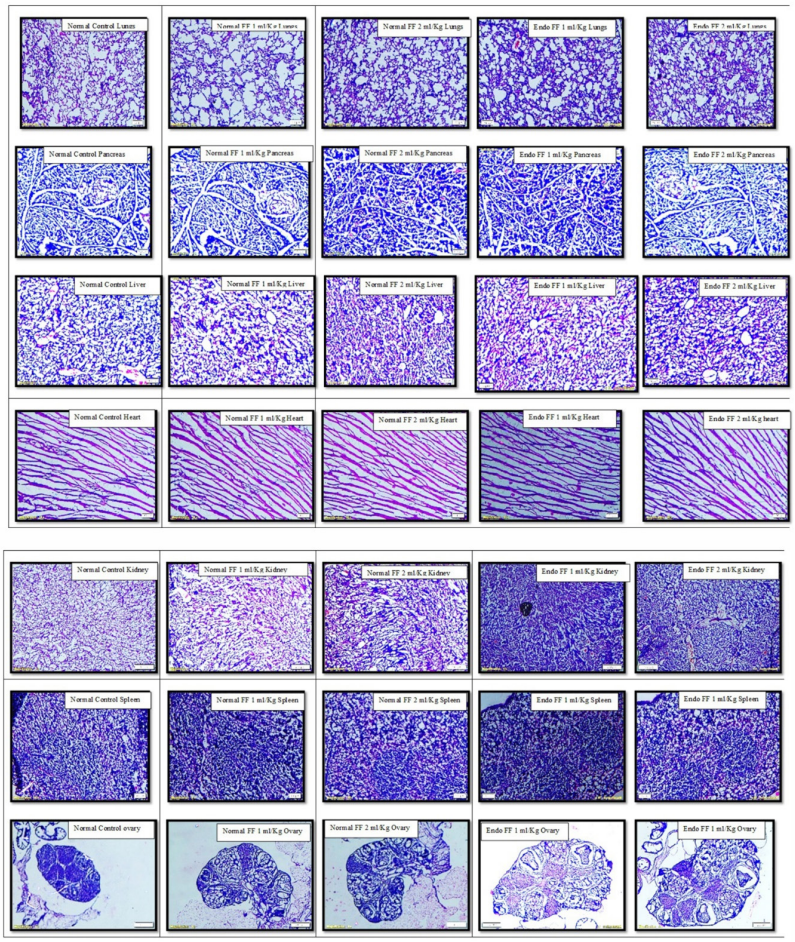

Figure 6: Effect on heart, liver, kidney, lung, pancreas, spleen and ovary, Histology of the heart, liver, kidney, lung, pancreas, spleen, and ovary of female mice given follicular fluid from normal persons in doses of 1 and 2 mL/kg intraperitoneally.

Insulin Resistance) also showed substantial significance ($p<0.001$) increase in insulin resistance from normal 0.17 to 0.57±0.02 and 0.71±0.04 at 1 and 2 mL/kg respectively. HOMA-Beta (homeostasis model assessment of β-cell dysfunction) normal level was 8.17±0.3 increased significantly ($p<0.001$) to 10.05±0.4 beta cell dysfunction. Nevertheless, QUICKI (quantitative insulin sensitivity check index) were both dosages dependently and highly substantially ($p<0.001$) decreased insulin sensitivity from 1.31±0.07 dose dependently to 0.9±0.01and 0.83±0.02 (Figure 5).

Effect on heart, liver, kidney, lung, pancreas, spleen and ovary

The histology of the heart, liver, kidney, lung, pancreas, spleen, and ovary of female mice given follicular fluid from normal persons in doses of 1 and 2 mL/kg intraperitoneally for 21 days showed no appreciable abnormalities. As shown in Figure 6, endometriotic patient follicular fluid at doses of 1 and 2 mL/kg intraperitoneally significantly affected vital and other organs. Vascular blockage and inflammatory cell infiltration in the pancreas cause edema and beta cell loss. There was liver necrosis, fatty liver, thickening of hepatocytes, central and peripheral vascular blockage, infiltration of inflammatory cells, and collagen deposition. In female mice treated with endometriotic follicular fluid, myocarditis and cardiac hypertrophy were clearly discernible. A significant amount of fibrosis was found along with abnormal kidney structure. The numerous cysts that were present in mice are evident from the ovary histology. Inflammatory cells, swelling of the alveolar sac, and alveolar membrane had heavily penetrated the lungs (Figure 6).

DISCUSSION

Our findings suggest that endometriotic fluid has adverse impact on body weight, glucose metabolism, insulin activity, hormone profile, and histopathology of vital organs. This proves that the endometriotic fluid not only has local effect but also impact the physiology, biochemistry and histopathology.

Results of our study shows that administration of endometriotic fluid intraperitoneally induce insulin resistance, pancreatic beta cell dysfunction and the compensatory hyperinsulinemia which are also the part of clinical abnormalities in endometriosis. However, not all endometriosis-affected women have compensatory hyperinsulinemia or are insulin-resistant.[20] The clinical signs of hyperandrogenemia, on the other hand, generally appear during adolescence in women with endometriosis and are largely characterized by an excess of androgens and a high LH level.[21] Similar to the results obtained in this study. Obesity and abdominal obesity are linked to higher levels of blood androgens and luteinizing hormone, which exacerbates the clinical traits of infertility and monthly irregularity.[22] Hence, the etiology and pathophysiology of endometriosis are influenced by a complicated interaction between body mass index, abdominal obesity, insulin resistance, androgen level, and LH level.[23] Results of our study endorses these outcomes as we have seen the higher level of serum testosterone upon 21 days treatment with endometriotic fluid. It is well established that endometriosis increases the risks for diabetes and cardiovascular disease in young women as a long-term complication, and it is also the leading cause of hyperandrogenism and female infertility. The results of our study demonstrated hyperglycemia upon administration of endometriotic fluid dose dependently. The risk of developing Impaired Glucose Tolerance (IGT) or Type 2 Diabetes Mellitus (T2DM) is two to five times higher in those with endometriosis compared to those of normal. According to previous research, we showed that treatment with endometriotic follicular fluid for 21 days dramatically increased glucose intolerance. No matter the dosage, we saw an insignificant drop in fasting blood glucose levels when compared to the control group when administered normal follicular fluid. In otherwise healthy follicular fluids, consistent effects on glucose tolerance can be seen. Normal fluid may therefore act as an insulin sensitizer, causing the Aberrant Glucose Tolerance (AGT) to switch to Normal Glucose Tolerance, which could influence the choice of endometriosis treatment (NGT) groups that received endometriotic follicular fluid treatment. Body weight was observed to be much more stable in the group receiving normal follicular fluid than in the control group.[24]

The serum estrogen level released by the dominant follicle is inversely correlated with success of ovulation. Comparing endometriosis-affected women to non-affected ones, androgen concentrations in follicular fluid are higher while estrogen concentrations are lower. According to our research, their systemic levels altered due to endometriotic 1 and 2 mL/kg of follicular fluid upon parenteral administration. Both estrogen and progesterone levels considerably reduced following endometriotic fluid administration which could account for the observed drop in the diestrus phase and compromise in estrous cycle regularity.[25,26] Several studies looking at women with endometriotic have demonstrated that high LH secretion facilitates overproduction of ovarian androgens, which in turn is connected to heightened pituitary sensitivity to GnRH. Our study demonstrates that 2 mL/kg of endometriotic follicular fluid significantly increases LH levels. As a result, the LH: FSH ratio rises, while in the normal follicular fluid 1 mL/kg and 2 mL/kg groups, it falls.

Normal follicular fluid administered to mice for 21 days caused considerable increase in RBC, platelet, hemoglobin, hematocrit, MHC, and P-LCR. Nonetheless, there was a noticeable decline in MCV and RDW-CV. The MCV, RDW-CV, RDW-SD, MPV, PCT, and P-LCR values were also dramatically raised after treatment with endometriosis follicular fluid. However, a number of variables, including hemoglobin, hematocrit, MHC, MCHC, and PCT, were significantly decreased (Table 1). Supporting the

fact that systemic effect of endometriotic disease cause an adverse impact on overall health of an individual.[27]

Figure 1 shows the results of a 21-day chronic endometriosis and normal follicular fluid treatment on female mice. On days 14 and 21, a normal follicular fluid therapy of 2 mL/kg resulted in a substantial ($p<0.05$) increase in body weight. On days 14 and 21, highly significant ($p<0.001$) weight loss in female mice was seen as a result of endometriosis fluid treatment. On days 14 and 21, a very significant ($p<0.01$) elevation was seen at a dose of 1 mL/kg compared to day 1. Female mice receiving parenteral endometriosis follicular fluid therapy experienced a highly significant ($p<0.001$) rise in blood sugar levels. It was discovered to be dose- and treatment-dependent. Endometriosis follicular fluid administration to female mice resulted in a dose-dependent drop in serum levels of estradiol and progesterone and an increase in testosterone (Figure 3). Normal follicular fluid considerably ($p<0.01$) lowered the level of progesterone in comparison, but there were no other significant changes in the levels of testosterone or estradiol. Parenteral endometriotic follicular fluid treatment elevated blood LH levels nearly three times the normal in female mice at doses of 1 and 2 mL/kg, highly substantially ($p<0.001$) (Figure 4). In contrast, the blood FSH level decreased at doses of 1 mL/kg and 2 mL/kg of endometriosis follicular fluid. Female mice were parenterally administered 1 and 2 mL/kg doses of endometriosis follicular fluid for 21 days in an effort to determine the impact on insulin activity and profile There was a dose-dependent rise in serum insulin level that was extremely significant ($p<0.001$). HOMA-IR and HOMA-Beta were similarly very significant ($p<0.001$) higher in a similar way. In contrast, QUICKI were dose-dependently and highly significantly ($p<0.001$) decreased.

Female mice were given follicular fluid from normal persons at doses of 1 and 2 mL/kg intraperitoneally (i.p) for 21 days, and we found no appreciable abnormalities in the histology of the heart, liver, kidney, lung, pancreas, spleen, and ovary. As shown in Figure 6, endometriotic patients; follicular fluid at doses of 1 and 2 mL/kg intraperitoneally significantly affected vital and other organs. Vascular blockage and inflammatory cell infiltration in the pancreas caused edema and beta cell death could be the cause for hyperglycemia and pancreatic beta cell dysfunction.[24] The presence of central and peripheral arterial occlusion, necrosis, fatty liver, thickening of hepatocytes, and collagen deposition in the liver were all identified similar to the metabolic dysfunction in endometriotic cases. Female mice treated with endometriosis follicular fluid had myocarditis and cardiac enlargement in an obvious manner. With severe fibrosis, abnormal kidney structure was also seen.[28] Supporting the fact that there is a chronic risk and complication for cardiovascular followed by compromise in kidney function. Several cysts were also present in the ovary of the mice, according to the histology of the organ. Inflammatory cells heavily entered the lungs, thickening the alveolar membrane and sac.[29]

This is the first study of its kind using follicular fluid obtained from healthy individuals and endometriotic patients, and it demonstrates that endometriotic complications are caused not only by a localized effect but also by a significant systemic effect on numerous organs and systems of the body. This demonstrates the viability of creating animal endometriosis models using endometriosis follicular fluid. The study should have, used a wider range of fluid dosages to observe the diversity and severity of systemic and histological effects.

CONCLUSION

Based on the results of present study it is concluded that endometriotic follicular fluid not only has a localized effect but also contains elements that enter the systemic circulation, cause negative effects, and possibly contribute to significant clinical endometriosis signs, symptoms and complications. The diagnostic and prognostic value of endometriotic follicular fluid should also be investigated. Using cutting-edge tools and methods, a study is currently underway in our laboratory to quantitatively assess the components of follicular fluid responsible for these effects.

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AUTHORS' CONTRIBUTIONS

Conceptualization, H.R.C. and N.G.; methodology, H.R.C., N.G., and R.S.; software, N.G.; validation, R.S. and H.R.C.; formal analysis, N.G. and H.R.C.; investigation, H.R.C., N.G., and R.S.; resources, H.R.C. and R.S.; data curation, N.G.; writing-original draft preparation, N.G. and H.R.C.; writing-review and editing, N.G., H.R.C., R.S.; visualization, H.R.C. and N.G.; supervision, H.R.C. and R.S.; project administration, H.R.C. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AGT: Aberrant glucose tolerance; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **EDTA:** Ethylene Diamine Tetra Acetate; **FF:** Follicular fluid; **FSH:** Follicle stimulating hormone; **GnRH:** Gonadotropin releasing hormone; **HOMA-Beta:** Homeostasis model assessment of β-cell function; **HOMA-IR:** Homeostatic Model Assessment

for Insulin Resistance; **IGT:** Impaired glucose tolerance; **IVF:** *In vitro* fertilization; **LH:** Leutinizing hormone; **LUF:** Leutinized unruptured follicle; **NSAID:** Nonsteroidal anti-inflammatory drugs (NSAIDs); **QUICKI:** Quantitative insulin-sensitivity check index; **MHC:** Mean Corpuscular Hemoglobin; **P-LCR:** Platelet-large cell ratio; **RBC:** Red blood corpuscles; **MCV:** Mean Corpuscular Volume; **RDW-CV:** Red Cell Distribution Width.

ETHICAL APPROVAL

Informed consent signed by the patient was obtained from all participants for this study. The institutional ethics committee of the DIT University of Dehradun, India, approved the study protocol on 10 January 2022, protocol number: 22-03-8 (Number of ethics approval: DITU/IAEC/22/03/08).

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