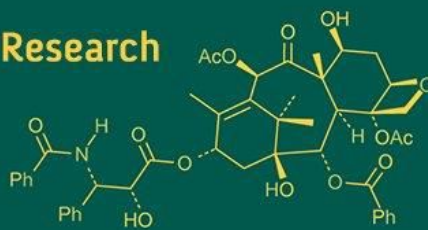


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(2): 469-479
www.biochemjournal.com
Received: 09-11-2024
Accepted: 18-12-2024

Mohit Pun
Punjab Agricultural
University, Ludhiana, Punjab,
India

Nisha Vashishat
Punjab Agricultural
University, Ludhiana, Punjab,
India

Ashman Sidhu
Punjab Agricultural
University, Ludhiana, Punjab,
India

Effects of quinnestrol treatment on regional protein expression in the rat testis and epididymis: Insights from SDS-Page, Western Blotting, and ELISA

Mohit Pun, Nisha Vashishat and Ashman Sidhu

DOI: <https://doi.org/10.33545/26174693.2025.v9.i2g.3829>

Abstract

This study investigated the effects of quinnestrol, a synthetic estrogen, on the male reproductive system of adult male albino rats, with a focus on sperm membrane proteins, particularly lactate dehydrogenase-C. Forty adult male albino rats were divided into two groups (n=20) after acclimatization: a control group receiving peanut/corn oil and a treatment group administered quinnestrol orally for 10 days at a dosage of 2 mg/kg of body weight. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of tissue homogenate of testis and epididymis, epididymal fluid and sperm membrane extract revealed reduced protein quality and band intensity in quinnestrol-treated rats as compared to controls. Notably, a 35 kDa band corresponding to lactate dehydrogenase-C, crucial for sperm function, was identified in sperm membrane extract. Western blotting confirmed reduced lactate dehydrogenase-C expression in sperm membrane extract from quinnestrol-treated rats, further corroborated by Enzyme-Linked Immunosorbent Assay. These findings indicate that quinnestrol disrupts biochemical parameters in the testis and epididymis, leading to decreased sperm protein quality and quantity, particularly affecting lactate dehydrogenase C, essential for sperm function.

Keywords: Quinnestrol, adult male albino rats, lactate dehydrogenase C, sodium dodecyl sulphate-polyacrylamide gel electrophoresis, western blotting, enzyme-linked immunosorbent assay

Introduction

The world's population has grown by 380 million people since the year 2000 to reach 6.5 billion. Despite reduced birth rates, the world population is estimated to reach 9.7 billion by the year 2050 and continue growing at a rate of 34 million people per year by the middle of the century, with the vast majority of these people living in developing nations (Leridon, 2020) [21]. The huge number of unwanted, usually adolescent pregnancies is another major issue. Over the last 50 years, there has been a steady improvement in contraceptive research. Despite considerable advancements in contraception alternatives for women over the last 50 years, the global population continues to rise at an alarming rate. Large-scale programs to meet the world's unmet family planning requirements have collected and driven scientists, healthcare providers, program managers, and users to re-examine the many techniques of contraception, focusing on those that have been demonstrated to be more effective (Sitruk-Ware *et al.*, 2013) [28]. Birth control is an essential means to regulate the population and control fertility rates so that it does not hinder the socio-economic growth of the nation.

Contraception is the deliberate prevention of pregnancy using various methods, including behavioral, pharmaceutical, and surgical techniques. Effective contraception enables couples to engage in sexual activity without the risk of unintended pregnancy while providing flexibility for family planning. Modern contraceptives influence estrogen and progesterone levels and include oral contraceptive pills, long-acting injections, implants, transdermal patches, and intrauterine devices (Hubacher & Trussell, 2015) [15].

Fertility regulation is categorized into hormonal and non-hormonal contraception. Hormonal contraceptives contain synthetic estrogen, such as ethinyl estradiol, or synthetic progestin, primarily in oral form but also available as implants and vaginal inserts (Colquitt & Martin, 2017) [6]. A common combination, quinnestrol and levonorgestrel in a 1:2 ratio, offers high efficacy with minimal side effects (Wright *et al.*, 2020) [34]. Progestin prevents ovulation by inhibiting the luteinizing hormone surge, thickening cervical mucus, reducing tubal motility,

Corresponding Author:
Mohit Pun
Punjab Agricultural
University, Ludhiana, Punjab,
India

and thinning the endometrial lining. Estrogen enhances effectiveness by suppressing follicle-stimulating hormone and potentiating progesterin's action on the luteinizing hormone surge (Kiley & Hammond, 2007; Dhont, 2010) [19, 9].

Quinestrol, a long-lasting, fat-soluble estrogen derivative of ethinyl estradiol, has been used in recent years to limit fertility by altering the structure and function of reproductive organs. It is a major long-term contraceptive taken by women for extended periods. Levonorgestrel and quinestrol, combined in a 2:1 ratio, are commercially used as female contraceptive medicines. Similar to its contraceptive efficacy in females, various steroid hormone combinations have been explored for male contraception, demonstrating potential antifertility effects. After gastrointestinal absorption, quinestrol is stored in adipose tissue and released gradually, contributing to its long-lasting impact. As a stable estradiol homolog, it suppresses the hypothalamic-pituitary-gonadal axis, reducing gonadotropin-releasing hormone secretion and impairing fertility regulation (James *et al.*, 2020) [17].

The hypothalamic-pituitary-gonadal axis is the primary endocrine feedback mechanism controlling reproductive function. Its suppression leads to decreased levels of follicle-stimulating hormone, luteinizing hormone, and testosterone or estradiol, which disrupts spermatogenesis and ovulation. While androgens and progesterone can reduce male fertility, high doses of estrogen and progesterone effectively inhibit female ovulation, suppressing follicle-stimulating hormone and luteinizing hormone production and preventing luteinizing hormone surges. Studies indicate that estrogen treatment has a stronger impact on male infertility compared to androgens, as it directly suppresses the hypothalamic-pituitary-gonadal axis, reduces testosterone levels, and obstructs normal spermatogenesis, ultimately leading to male infertility (Dacheux & Dacheux, 2014) [8]. Xenoestrogens, which mimic estrogen, further reduce male fertility by disrupting Leydig cell function, either directly or through indirect effects on the hypothalamic-pituitary-gonadal axis (Sullivan & Mieusset, 2016) [29].

Research suggests that estrogen could be an effective agent for fertility control in male rats. Xenoestrogens have been linked to testicular hypoplasia, rete testis tumors, and erectile dysfunction. Quinestrol, apart from being a human contraceptive, is also used as a rodenticide to control wild rat populations. High doses of quinestrol have been shown to induce antifertility effects in laboratory rodents. Various xenoestrogens, including diethylstilbestrol, estradiol-17, and phytoestrogens, have been reported to reduce testosterone production in rodents, birds, and mammals, leading to decreased fertility. Early studies on orally active estrogens, progestagens, and androgens demonstrated significant disruptions in ovulation, implantation, and spermatogenesis. Quinestrol alone or in combination alters reproductive organ structures, reducing male fertility in rats and wild rodents. Several laboratory and field studies confirm that the compound EP-1 effectively decreases fertility in multiple wild rodent species (Chakraborty & Saha, 2022) [3].

The antifertility effects of quinestrol are primarily due to oxidative stress, germ cell apoptosis, and reduced plasma testosterone levels. Any disturbance in the hypothalamic-pituitary-testicular axis, which regulates sexual

differentiation, development, and fertility, can lead to male gonadal dysfunction.

Spermatogenesis commences in the seminiferous tubules of the testis and begins at the basal membrane and continues to the tubule lumen via the outermost portion of the Sertoli cells that border the seminiferous tubules (Reisse *et al.*, 2001) [25]. It is a continuous process in males by which mature and functionally active spermatozoa are produced in male gonads. Spermatozoa that leave the testis and enter the epididymis, a long convoluted tubule, are non-functional gametes (Gervasi & Visconti, 2016) [11]. The epididymis is a crescent-shaped, convoluted part of the male reproductive system, a long tube that links male gonads to the male duct, the vas deferens, and it is derived from the Wolffian duct and is mostly made up of mesenchymal tissue during birth (Turner, 2008) [31]. It is partitioned into three primary segments: caput, corpus, and cauda, each of which is further divided into intra-segmental zones delimited by connective tissue septa and expresses a distinct pattern of genes (Sullivan & Mieusset, 2016) [29].

Understanding the qualitative and quantitative changes in tissue homogenate of testis and epididymis, epididymal fluid and sperm membrane extract (SME) is crucial for elucidating the molecular mechanisms underlying quinestrol-induced infertility. Therefore, this study aims to determine the molecular changes in spermatozoa during the maturation process in response to quinestrol exposure using advanced biochemical techniques such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting, and enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

The procurement, acclimatization, and maintenance of male albino rats were conducted in strict compliance with ethical and regulatory standards to ensure the welfare of the animals and the reliability of the study. Proper housing conditions, controlled environmental factors, and a balanced diet were maintained to minimize stress and variability. Institutional approvals and adherence to CPCSEA guidelines reinforced the ethical integrity of the research. By following these protocols, the study upheld scientific rigor while ensuring humane treatment, contributing to the validity and reproducibility of the experimental outcomes.

Drug used for the experiment

To evaluate the effects of quinestrol, synthetic estradiol (17 alpha-Ethinylestradiol 3-cyclopentyl ether, $C_{20}H_{32}O_2$, 364 g/mol) a commercial formulation was acquired from CDH Chemicals and administered to the rats by oral intubation.

Dose used in the experiment

Rats were acclimated to lab conditions for a period of fifteen days prior to the experiment.

All 40 male rats were separated into two groups (n=20) after acclimatization.

Group 1: Control

Group 2: 2mg/kg quinestrol body weight

Every dosage that contains quinestrol dissolves in Dimethyl sulphoxide because quinestrol dissolves in an organic solvent. For ten days, oral intubation was used as the primary mode of treatment.

Autopsy of rats

After the course of treatment was completed, the rats were dissected. The testicles and epididymides were taken out and their adhering tissue was removed in order to weigh them precisely.

Extraction of Caput, Corpus, and Cauda Sperm Membrane Proteins

The membrane proteins of caput, corpus, and cauda sperm were extracted using SDS as described by Jones *et al.* (1983) [35]. After two washes with PBS (pH 7.4), spermatozoa (13×10^6) were suspended in 1.0 ml of 15 mM DCC/2% SDS in 62.5 mM Tris-HCl (pH 8.0), containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 25 mM benzidine, and 10 mM aprotinin). The sperm suspensions were sonicated three times at 20 watts for 20 seconds, followed by centrifugation at 10,000 g for 30 minutes to collect the supernatant. This process was repeated twice, first with centrifugation at 20,000 g for 30 minutes and then at 10,000 g for 45 minutes. The SMEs were collected from the pellets and stored in 0.1 ml aliquots at -20°C until needed.

Preparation of testis and epididymal tissue homogenates for SDS-PAGE and fluid collection from caput, corpus, and cauda regions of the epididymis.

The testis and epididymal tissues, including the caput, corpus, and cauda regions, were homogenized by combining the tissue with a known quantity of 0.1 M phosphate-buffered saline (pH 7.6). The homogenization process was carried out using a tissue homogenizer. The entire homogenate was then centrifuged in a chilled microcentrifuge at 3000 revolutions per minute (825 relative centrifugal force) for 10 minutes. The tissue homogenates of the testis and epididymis were collected for further studies. Similarly fluid was collected from three regions of the epididymis i.e. caput, corpus and cauda for further studies.

Protein Extraction from Animal Tissues (Cilia *et al.*, 2009) [5]

Trichloroacetic acid (TCA)-acetone method was used for the extraction of proteins from testis and epididymis tissues. Frozen tissue was homogenized in ice-chilled PBS, and the homogenate was then mixed with 10% TCA in 10 ml acetone containing 2% beta-mercaptoethanol. The mixture was stirred by inverting the tube ten times and incubated at -20°C for at least 12 hours to facilitate protein precipitation. The precipitated protein was centrifuged at 5000 g for 30 minutes. The resulting pellet was washed ten times with 10 ml of ice-cold acetone, with vigorous disruption using a crusher between washes. The protein was then air-dried and stored at -20°C until further use.

Sample Preparation for SDS-PAGE using TCA Precipitation

The extract was mixed with TCA at a final concentration of 10-20% and incubated on ice for 30 minutes for protein precipitation. The sample was then centrifuged at 14,000 rpm for 30 minutes at 4°C in a microcentrifuge. The pellet was then washed by centrifuging again at 14,000 rpm for 10 minutes, followed by draining the acetone. The washing step was repeated, and any residual acetone was removed by air drying. The dried sample was resuspended in 20-30 µl of sample buffer containing β-mercaptoethanol, heated at 95°C

for 1-2 minutes, and briefly spun in a table centrifuge. The sample was then ready for SDS-PAGE analysis.

Protein qualitative analysis using SDS-PAGE (Laemmli, 1970) [20]

SDS-PAGE was performed following the method described by Laemmli (1970) [20]. Resolving and stacking gels were prepared using electrophoresis apparatus. Protein samples (20 µg) were mixed with sample buffer and heated in boiling water for 5-10 minutes before being loaded into the wells of the gel. The electrophoresis apparatus was run, and the gel was stained with 0.5% Coomassie Brilliant Blue R-250 solution for 20-30 minutes. The gel was then destained using a solution containing 20% methanol, 10% glacial acetic acid, and 70% distilled water, with multiple changes of the destaining solution until distinct protein bands were visible. The molecular weight of protein bands in different lanes was recorded in kilodaltons (kDa) after comparison with a protein marker.

Detection of sperm membrane proteins by western blotting (Towbin *et al.*, 1979) [30]

Membrane proteins from caput, corpus, and cauda-SME were identified using western blotting. Proteins from cauda-SME were separated by SDS-PAGE and transferred onto a nitrocellulose membrane (NCM) using a blot sandwich setup in transfer buffer. Transfer was performed at 100 V for 2-3 hours with cooling pads to prevent overheating. Transfer quality was assessed with Ponceau Dye, followed by washing. The membrane was blocked overnight at 4°C, washed with phosphate buffer saline (PBS), and incubated with the primary antibody for 2-3 hours at 37°C. After washing, it was incubated with HRPO-conjugated anti-rat IgG (1:10,000) for 2-3 hours, followed by further washing. Detection was performed using a substrate solution containing DAB, 4-chloronaphthol, and H₂O₂ for 10 minutes. The reaction was stopped by washing with distilled water.

Detection/Titration of Anti-Cauda SDS-SME Antibodies by ELISA

LDH-C like proteins in tissue and SMEs from the epididymis were quantified using indirect ELISA (Crowther, 1995) [7]. A 96-well ELISA plate was coated with 100 µl of antigen (2 µg/100 µl) and incubated at 37°C for 3 hours, followed by PBS washing. Blocking was done with 2% BSA for 2 hours at 37°C, then washed again. Anti-LDH-C antibody was added and incubated overnight at 4°C, followed by PBS washing. HRPO-conjugated anti-rat IgG (1:10,000) was added and incubated for 3 hours at 37°C, followed by washing. The reaction was developed with OPD-H₂O₂ substrate for 20 minutes in the dark, stopped with 5N H₂SO₄, and absorbance was measured at 492 nm using an ELISA reader.

Statistical Analysis

All values are expressed as mean±S.E. Statistical Package for Social Sciences (SPSS) version 16 for Windows was used to determine significant differences among the groups i.e., control and group treated with 2 mg/kg b.wt. of quineestrol using t-test (independent), where if value of p< 0.05, the values are said to differ significantly.

Results

Qualitative analysis of proteins by SDS-PAGE in homogenates of testis and epididymal tissues

The SDS-PAGE (figure 1) indicated the presence of 6 bands corresponding to molecular weight of 70, 67, 45, 35, 24, 17 kDa proteins in homogenate of testis; 5 bands corresponding to molecular weight of 70, 55, 40, 35, 15 kDa proteins were present in caput; 5 bands corresponding to molecular weight of 70, 55, 35, 25, 15 kDa proteins were present in corpus and 6 bands corresponding to molecular weight of 70, 67, 55, 35, 25, 15 kDa proteins were present in cauda. All of these bands were reported in control rats. Similarly, 6 bands corresponding to molecular weight of 70, 67, 45, 35, 24, 17 kDa proteins were present in testis; 4 bands corresponding to molecular weight of 70, 48, 35, 15 kDa proteins were present in caput; 3 bands corresponding to molecular weight of 70, 35, 15 kDa proteins were present in corpus and 5 bands corresponding to molecular weight of 70, 67, 55, 35, 25 kDa proteins were present in cauda. All of these bands were present in rats treated with quineestrol for a period of 10 days. The variation in proteins table 2-5. Standard molecular marker is given in table 1 for comparison with treated group. This revealed that treatment with quineestrol affects processes of protein synthesis in male albino rats. Also, oxidative stress can raise free radical levels in cells, which leads to protein oxidation. Proteins oxidise when their molecules interact with the radicals. This causes the protein's structure to alter and become aberrant, further this indicates inhibition of transcription and translation, eventually resulting in loss of some proteins (Hayati *et al.* 2010) [16].

Qualitative analysis of proteins by SDS-PAGE of epididymal fluid of three regions of epididymis

The SDS-PAGE analysis of epididymal fluid from control and quineestrol-treated rats revealed significant changes in protein profiles across the caput, corpus, and cauda regions of the epididymis. The trend of height, raw volume and % raw volume in protein files between control and treated rats were presented in table 6-8 and figure 2. In control rats, proteins with molecular weights of 100, 80, 55, 40, 20, and 14 kDa were observed, whereas treated rats exhibited proteins of 80, 55, 40, 35, 30, and 25 kDa. Notably, proteins of 20 and 14 kDa were absent in treated rats, while new bands corresponding to 30, 25, and 35 kDa appeared in the epididymal fluid of treated rats. This suggests that quineestrol administration alters the protein composition of the epididymal fluid, potentially due to oxidative stress-induced damage or disruptions in the epididymal microenvironment, which is critical for sperm maturation (Shen *et al.*, 2011; Jones, 1998) [26, 18].

Qualitative analysis of proteins by SDS-SMEs of three regions of epididymis

The SDS-PAGE indicated the presence 6 proteins corresponding to molecular weight of 70, 57, 40, 36, 35, 25 kDa in the SME of caput, corpus and cauda epididymal fluid of control rats and similarly 70, 57, 40, 36, 35, 25 kDa proteins were observed in the group treated with quineestrol. The trend of height, raw volume and % raw volume in protein files between control and treated rats were presented in table 9-11 and figure 3. In the present study it has been reported that quality and quantity of sperm membrane proteins have been decreased after quineestrol treatment.

Detection of Lactate dehydrogenase-C proteins by western blotting

The SDS-PAGE indicated the presence of 70, 57, 40, 36, 35, 25 kDa proteins in the SME of caput, corpus and cauda of control rats and similarly 70, 57, 40, 36, 35, 25 kDa proteins were observed in the group treated with quineestrol @ 2 mg/Kg body weight for a period of 10 days. The results of SDS-PAGE of SME of three regions of indicated that protein quantity decreases in comparison to control group. The 35 kDa protein band was observed in tissue homogenate of testis and epididymis and in SME of control and treated rats but this band was absent in epididymal fluid of control and treated rats. The presence of this protein in testis and SME indicated that after secreting from tissue it might get absorbed on the surface of sperm in the epididymis. In SME of three regions of epididymis a 35 kDa protein was identified which had remarkable role in male fertility. The results on the NCM membrane (figure 4) showed that the quantity of the targeted protein i.e. LDH-C after reaction with selected antibody in the caput, corpus and SME decreases after quineestrol as compared to the control group.

Determination of LDH-C concentration by ELISA

Western blotting results indicated the presence of 35 kDa protein in caput, corpus and cauda SME of control and treated rats. In the present study, the characteristic expression of particular protein i.e LDH-C was analysed in extract of testis and SME of caput, corpus and cauda using ELISA. The results are depicted in table 12 and figure 5. Quantity of proteins was estimated from the absorbance at 492 nm and was calculated by determining the per cent change.

The per cent age change in testis of control and treated rats was 0.73 ± 0.12 and 0.55 ± 0.09 . Similarly, per cent age change in caput, corpus and cauda of control and treated rats was 0.21 ± 0.09 , 0.18 ± 0.05 , 0.68 ± 0.14 and 0.15 ± 0.07 , 0.11 ± 0.01 , 0.41 ± 0.08 . The data showed that there was significant difference between the control and treated rats. This revealed that quantity of LDH-C decreases after quineestrol treatment in male albino rats. Similar trend was observed in quantity of caput, corpus and cauda which indicated the quantity in the treated rats decreases after quineestrol treatment.

Discussion

The present study highlights the profound effects of quineestrol on protein expression in the male reproductive system, particularly in the testis and epididymis. The SDS-PAGE analysis revealed significant alterations in protein band patterns between control and quineestrol-treated rats, indicating that quineestrol affects protein synthesis, degradation, and expression, ultimately disrupting critical processes involved in spermatogenesis, sperm maturation, and fertilization.

The findings demonstrate that while certain testicular proteins (70, 67, 45, 35, 24, and 17 kDa) remained consistently present in both control and treated rats, epididymal protein expression was significantly altered by quineestrol. Notably, the 55 kDa protein band was absent in the caput and corpus epididymis of treated rats, suggesting disruption in sperm maturation. Additionally, the loss of the 15 kDa band in the cauda epididymis highlights quineestrol's potential adverse effects on sperm function.

A critical observation was the reduced intensity and raw volume of the 67 kDa protein band, corresponding to estrogen receptor- α (ER α). ER α is crucial for testicular function and sperm viability, and its downregulation in quinestril-treated rats may contribute to impaired spermatogenesis and steroidogenesis (Hess *et al.*, 1997; Cavaco *et al.*, 2009) [14, 2].

Similarly, decreased expression of the 45 kDa band, associated with acrosomal protein SP-10, suggests a potential impairment in sperm-egg interaction and fertilization (Naz & Vanek, 1998; Batista *et al.*, 2022) [23, 1]. The absence of the 55 kDa band, corresponding to sperm-associated fertilin- β , in the caput and corpus further supports the hypothesis that quinestril impairs sperm-egg fusion (Cho *et al.*, 1998; Gundogan *et al.*, 2022) [4, 13].

The epididymal fluid analysis revealed substantial protein modifications, with the appearance of new proteins (30 and 25 kDa in the caput, 30 kDa in the corpus, and 35 and 25 kDa in the cauda) in treated rats. Conversely, the loss of 20 and 14 kDa proteins across all epididymal regions suggests that quinestril disrupts the normal protein acquisition process essential for sperm maturation. These changes are consistent with oxidative stress-induced protein oxidation, which damages reproductive organ membranes and interferes with sperm-epididymal interactions (Shen *et al.*, 2011) [26].

Epididymal secretory proteins are essential for sperm function, with previous studies highlighting their role in sperm motility and fertilization (Veron *et al.*, 1982; Gonzalez *et al.*, 1984; Jones, 1998) [32, 12, 18]. Quinestril-induced alterations in the epididymal microenvironment may result in significant disruptions to these processes, negatively impacting fertility. The decreased presence of key sperm membrane proteins, particularly those involved in sperm-egg binding, further supports the hypothesis that quinestril compromises male fertility by altering sperm membrane composition.

A significant decrease in sperm membrane proteins, particularly LDH-C (~35 kDa), was observed following

quinestril treatment. LDH-C is crucial for glycolysis, providing ATP necessary for sperm motility (Farhana & Lappin, 2022) [10]. Its downregulation aligns with previous studies linking impaired LDH-C function to male infertility (Odet *et al.*, 2011; Wang *et al.*, 2013) [24, 33]. Furthermore, the significant reduction in the 36 kDa protein band, identified as 17 β -hydroxysteroid dehydrogenase (17 β -HSD), suggests that quinestril disrupts androgen biosynthesis, leading to reduced testosterone levels and impaired spermatogenesis (Mindnich *et al.*, 2004; Sidhu *et al.*, 2020) [22, 27].

The reduction in aromatase (~57 kDa) expression in quinestril-treated rats is another critical finding. Aromatase plays a vital role in converting androgens to estrogens and is necessary for testicular homeostasis (Carreau *et al.*, 2003). Previous studies have demonstrated that aromatase inhibition results in impaired sperm function and reduced fertility (Luconi *et al.*, 2002; Rochira & Carani, 2009). The decreased aromatase levels in quinestril-treated rats further support the hypothesis that quinestril disrupts the androgen-estrogen balance, leading to infertility.

Western blot and ELISA analyses confirmed a significant decrease in LDH-C levels in the testis, caput, corpus, and cauda of treated rats. Given LDH-C's exclusive expression in the testis and its essential role in sperm metabolism, its downregulation may serve as a potential mechanism underlying quinestril-induced infertility. This suggests that LDH-C could be a key target for contraceptive interventions.

Overall, the findings of this study provide strong evidence that quinestril induces significant disruptions in male reproductive protein expression, affecting critical enzymes, receptors, and sperm maturation proteins.

These alterations likely contribute to impaired spermatogenesis, reduced sperm motility, and compromised fertilization ability. Future studies should further explore the molecular mechanisms underlying these disruptions and evaluate the long-term reproductive consequences of quinestril exposure.

Table 1: Analysis of protein bands of standard molecular marker

Molecular weight	Height	Raw Vol	% Raw vol
180	239.6821	53457.31	27.805018
130	223.5188	53923.804	20.328523
100	201.7552	65970.123	19.631948
70	229.2729	49194.551	17.186218
55	237.7083	59356.878	17.936231
40	133.7688	56172.887	18.325347
35	65.35292	55601.311	12.211865
25	77.27084	59164.625	19.192075
15	300.7906	532431.41	33.93609
10	95.96146	56331.75	22.384575

Table 2: Characteristics of proteins of testis of control and treated rats separated after SDS-PAGE analysis

Molecular weight kDa	Height		Raw volume		%Raw volume	
	C	T	C	T	C	T
70	112.1852	91.6642	41567.31	1232.12	2.87852	0.135658
67	33.09658	13.0185	31232.22	22364.2	8.3152	1.263232
45	11.78548	4.05823	36033.26	71151.35	2.42163	1.96852
35	12.53256	9.50636	18239.24	12756.2	1.96852	0.9852
24	11.70852	8.25653	36232.33	23253.5	10.2985	7.3254
17	3.25251	1.15236	33832.22	26098.22	4.2352	2.3565

C- Control group, T- Treatment group

Table 3: Characteristics of proteins of caput of control and treated rats separated after SDS-PAGE analysis

Molecular weight (kDa)	Height		Raw Volume		% Raw Volume	
	C	T	C	T	C	T
70	19.2355	17.6205	567.025	120.35	5.6545	4.2556
55	18.8622	---	100.563	---	10.2545	---
48	20.2682	11.2251	102.554	99.652	6.843	3.5845
35	16.6515	9.2316	709.255	15.885	1.9752	1.25345
15	11.7222	4.2555	732.256	140.255	8.312	2.89752

C- Control group, T- Treatment group, (---)- indicated no band

Table 4: Characteristics of proteins of corpus of control and treated rats separated after SDS-PAGE

Molecular weight (kDa)	Height		Raw Volume		% Raw Volume	
	C	T	C	T	C	T
70	8.73125	5.25268	19555.8	14563.12	2.58965	1.25623
55	35.4095	---	13697.2	---	1.963558	---
35	91.5632	35.59123	15515.23	11253.25	16.2555	8.2635
25	60.5000	---	23225.26	---	5.69851	---
15	36.0051	11.25	35321.25	15635.23	3.25955	4.25653

C- Control group, T- Treatment group, (---)- indicated no band

Table 5: Characteristics of proteins of cauda of control and treated rats separated after SDS-PAGE

Molecular weight (kDa)	Height		Raw Volume		% Raw Volume	
	C	T	C	T	C	T
70	66.1022	22.888	36056.24	1925.32	4.6325	1.2355
67	12.366	2.355	2325.652	1452.23	1.235	0.255
55	312.23	142.36	34186.5	24156.23	15.055	12.255
35	4.2255	3.2155	5535.23	1056.32	16.235	11.2565
25	12.3255	7.26632	6347.25	1123.25	1.2533	0.225
15	9.3265	---	4542.32	---	2.3636	---

C- Control group, T- Treatment group, (---)- indicated no band

Table 6: Characteristics of proteins of caput epididymal fluid of control and treated rats separated after SDS-PAGE

Mol weight (kDa)	Height		Raw volume		% Raw volume	
	C	T	C	T	C	T
100	213.125	---	7223.145	---	2.2543	---
80	33.256	23.563	50652.32	45236.25	1.893322	0.2556
55	180.256	122.365	49160.23	42856.32	2.5632	1.2563
40	61.256	36.564	31952.23	22541.3	1.5963	1.3652
30	---	38.295	---	1236.25	---	2.6452
25	---	25.626	---	1136.62	---	1.6528
20	18.662	---	15632.65	---	3.5555	---
14	55.265	---	14253.55	---	2.2544	---

C- Control group, T- Treatment group, (---)- indicated no band

Table 7: Characteristics of proteins of corpus epididymal fluid of control and treated rats separated after SDS-PAGE

Mol weight (kDa)	Height		Raw volume		% Raw volume	
	C	T	C	T	C	T
100	53.236	---	51146.26	---	4.2356	---
80	70.263	16.125	8962.215	4523.25	2.589	1.6532
55	33.1563	22.253	4020.325	3325.36	1.2533	0.2325
40	36.2563	16.585	4021.23	2263.55	2.5463	1.963
30	---	18.265	----	6235.63	----	2.352
25	---	15.268	---	3251.26	---	3.255
20	62.325	---	3196.54	---	10.235	---
14	15.026	---	7965.53	---	1.5235	---

C- Control group, T- Treatment group, (---)- indicated no band

Table 8: Characteristics of proteins of cauda epididymal fluid of control and treated rats separated after SDS-PAGE

Mol weight (kDa)	Height		Raw volume		% Raw volume	
	C	T	C	T	C	T
100	71.2635	---	4226.32	---	1.9632	---
80	65.123	45.365	1224.36	1125.23	1.6325	0.2563
55	34.236	22.655	5963.25	4423.68	2.2563	1.2563
40	9.255	9.2635	4146.25	3365.23	12.256	4.23645
35	---	12.366	---	2236.61	---	3.5264
25	---	16.256	---	1526.32	---	1.6532
20	69.655	---	22823.23	---	2.6533	---
14	17.6165	---	83526.23	---	1.6521	---

Table 9: Characteristics of proteins of SMEs of caput of control and treated rats separated after SDS-PAGE

Mol weight (kDa)	Height		Raw volume		% Raw volume	
	C	T	C	T	C	T
70	83.266	14.2552	48895.23	10843.25	17.69023	8.7822
57	156.325	93.5635	52158.26	38124.1	11.4693	1.5215
40	166.325	99.632	9120.854	3000.12	27.3956	11.652
36	98.3222	17.262	16639.25	12278.23	8.00153	6.5322
35	43.125	18.256	30597.23	22211.85	3.18325	2.8565
25	83.255	53.662	29254.32	15325.25	5.9983	4.2325

C- Control group, T- Treatment group

Table 10: Characteristics of proteins of SMEs of corpus of control and treated rats separated after SDS-PAGE

Mol weight (kDa)	Height		Raw volume		% Raw volume	
	C	T	C	T	C	T
70	64.655	23.5463	46953.23	42356.63	2.8562	1.8262
57	95.633	44.663	14800.26	11256.32	16.2555	10.2563
40	75.266	66.235	13552.22	86552.23	15.035	4.254
36	45.366	36.563	91250.36	75235.55	6.2153	3.6588
35	64.235	35.265	26693.55	11235.63	33.655	22.599
25	65.255	45.655	23243.42	12635.36	11.512	2.8565

C- Control group, T- Treatment group

Table 11: Characteristics of proteins of SMEs of cauda of control and treated rats separated after SDS-PAGE

Mol weight (kDa)	Height		Raw volume		% Raw volume	
	C	T	C	T	C	T
70	64.325	23.544	51953.5	49842.32	2.5633	1.8262
57	95.266	44.653	14802.26	96325.23	16.2558	10.2563
40	45.632	25.555	91250.3	88655.2	6.2153	3.6588
36	60.253	27.256	26693.55	1463.653	3.9525	22.599
35	65.145	60.255	23243.56	11236.23	11.512	2.8565
25	136.25	96.542	16331.75	13221.55	3.1828	2.6551

C- Control group, T- Treatment group

Table 12: Per cent change in mean absorbance after treatment with quinestril

Organs	Mean Absorbance of control samples	Mean Absorbance of treated samples	Per cent change
Testis	0.73±0.12	0.55±0.09	24.44%
Caput	0.21±0.09	0.15±0.07	28.57%
Corpus	0.18±0.05	0.11±0.01	38.80%
Cauda	0.68±0.14	0.41±0.08	39.00%

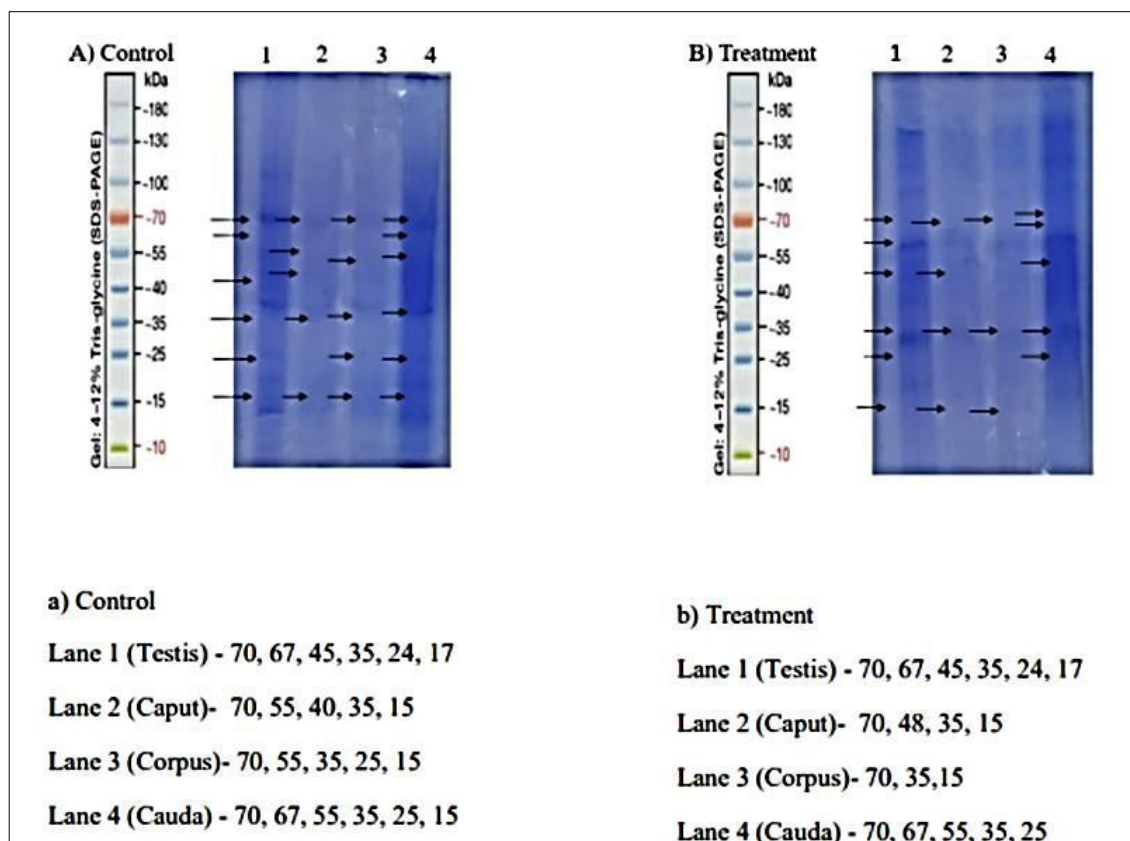


Fig 1: Qualitative analysis of proteins by SDS-PAGE in tissue homogenate of the testis and epididymis of a) control and b) treated rats

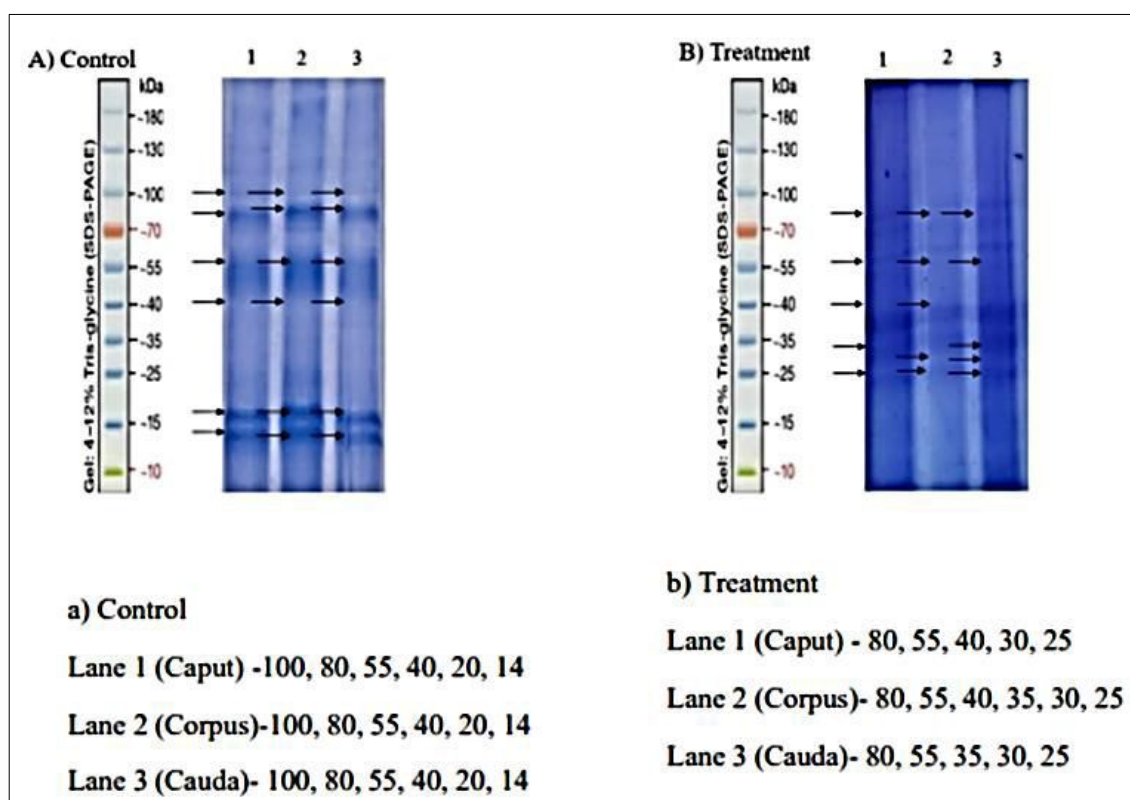


Fig 2: Qualitative analysis of proteins by SDS-PAGE of epididymal fluid of three regions of epididymis of a) control and b) treated rats

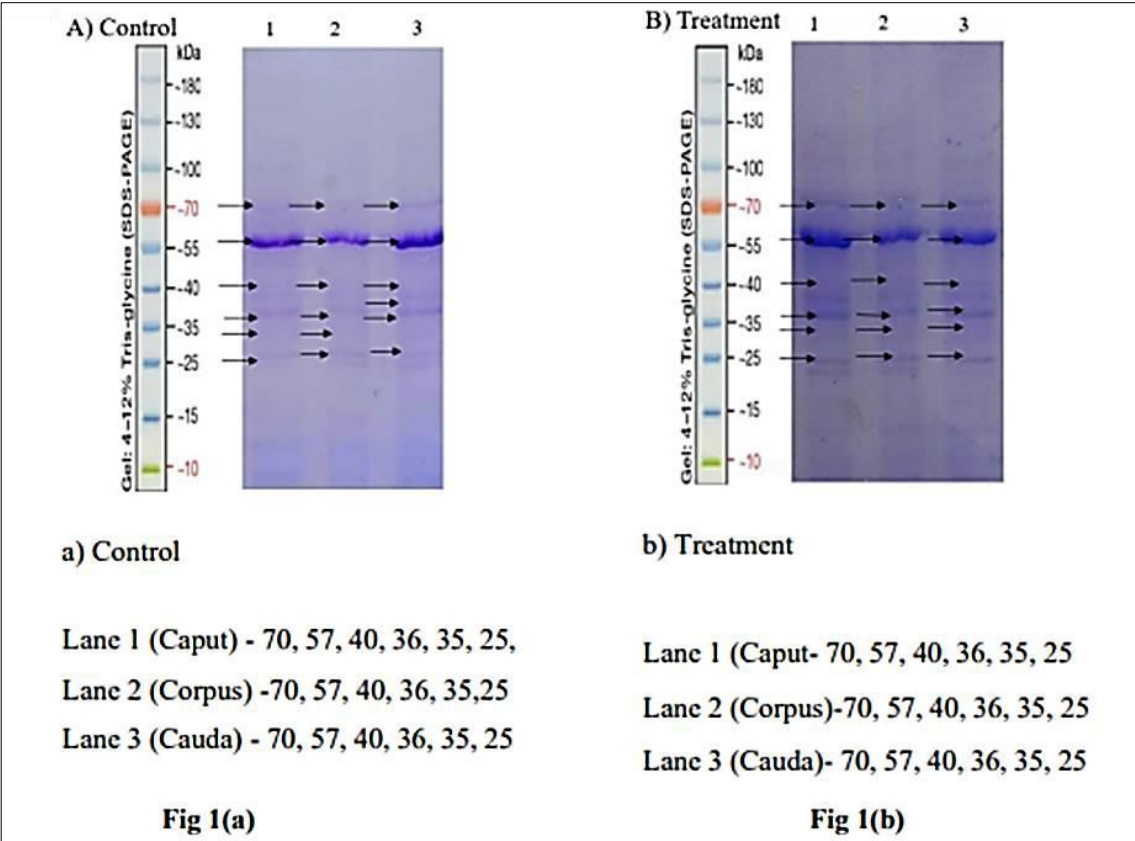


Fig 3: Qualitative analysis of proteins by SDS-PAGE of SMEs of three regions of the epididymis a) control b) treatment

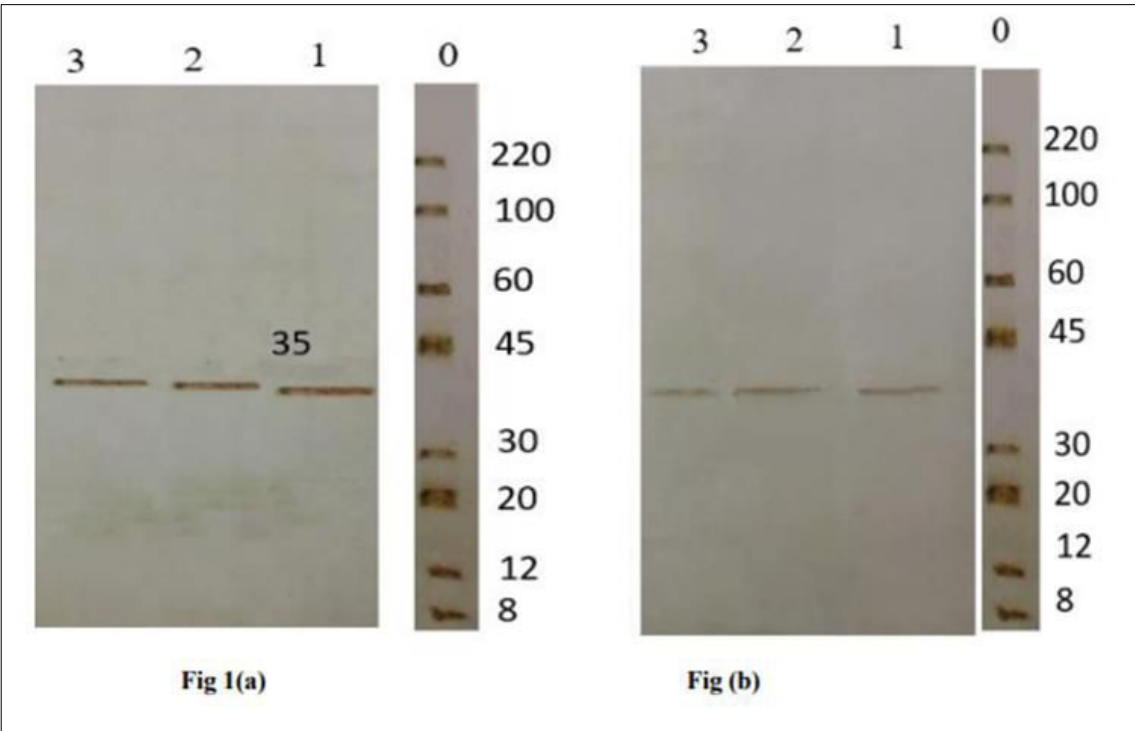


Fig 4: Western blotting of caput, corpus and cauda SMEs of male albino rats on nitrocellulose membrane a) control b) treatment

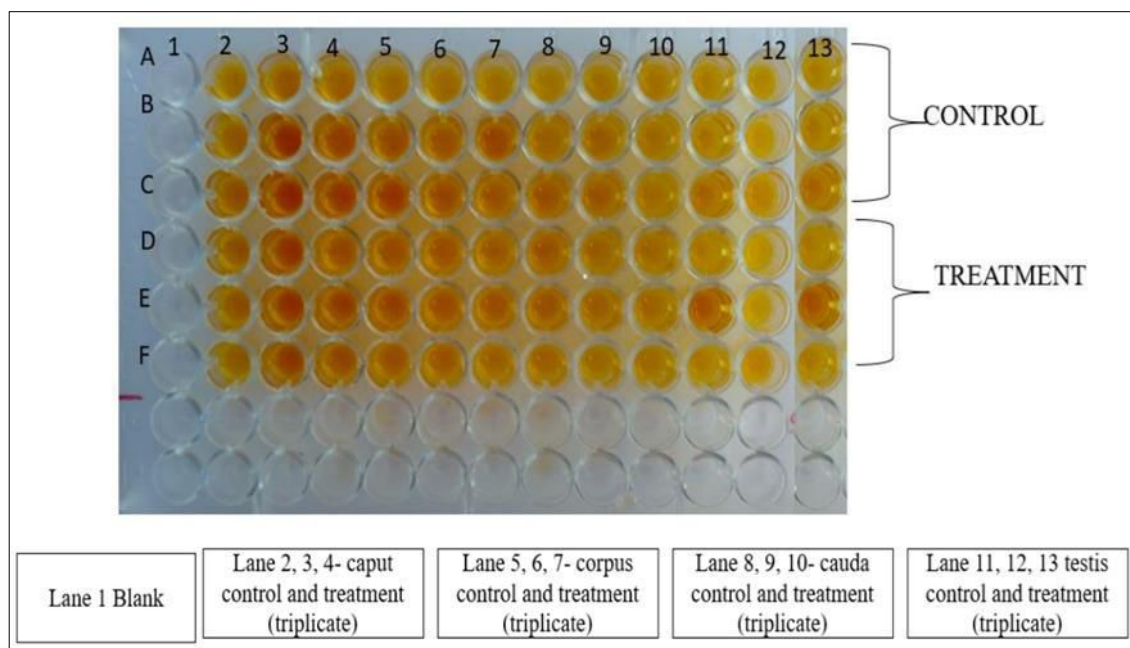


Fig 5: ELISA plate showing antigen antibody reaction in the wells.

Conclusion

This study demonstrates that quineestrol significantly disrupts key protein expression in the male reproductive system of albino rats. Alterations in proteins essential for spermatogenesis, sperm maturation, and fertilization—including ER α , SP-10, fertilin- β , LDH-C, 17 β -HSD, and aromatase—suggest a complex mechanism of action. The disruption of epididymal fluid protein composition and reduced LDH-C expression indicate impaired sperm maturation and motility. Additionally, disturbances in steroidogenic enzymes suggest hormonal imbalances. These findings highlight quineestrol's antifertility effects and its interference with critical protein pathways, warranting further research into its molecular mechanisms and long-term reproductive consequences.

References

1. Batista M, Rando G, Santini SJ, *et al.* The role of acrosomal proteins in sperm function and fertilization. *Reproductive Biology and Endocrinology*. 2022;20(1):45-58.
2. Cavaco JE, Laurentino SS, Gavaia PJ, *et al.* Estrogen receptors and testicular function: A role for ER α and ER β in male fertility. *Molecular and Cellular Endocrinology*. 2009;304(1-2):10-17.
3. Chakraborty S, Saha S. Quineestrol-induced reproductive alterations in wild rodent populations. *Journal of Wildlife Research*. 2022;48(2):135-147.
4. Cho C, Ge H, Branciforte D, *et al.* Fertilin β is required for sperm-egg membrane fusion. *Nature*. 1998;395(6701):402-405.
5. Cilia E, *et al.* Protein extraction methods in reproductive tissue analysis. *Journal of Biochemistry*. 2009;256(2):147-153.
6. Colquitt L, Martin J. Hormonal contraceptives: Mechanisms and efficacy. *International Journal of Reproductive Medicine*. 2017;26(3):215-230.
7. Crowther JR. ELISA: Theory and Practice. *Methods in Molecular Biology*. 1995;42(1):149-167.
8. Dacheux JL, Dacheux F. New insights into epididymal function in relation to sperm maturation. *Reproduction*. 2014;147(2):R27-R42.
9. Dhont M. History of oral contraception. *European Journal of Contraception & Reproductive Health Care*. 2010;15(2):57-66.
10. Farhana A, Lappin SL. Lactate dehydrogenase (LDH-C) and its role in sperm metabolism. *Journal of Andrology*. 2022;43(3):199-211.
11. Gervasi MG, Visconti PE. Molecular changes and signaling pathways involved in sperm capacitation. *Journal of Andrology*. 2016;37(5):485-498.
12. Gonzalez E, *et al.* Epididymal secretory proteins and sperm maturation. *Journal of Reproductive Fertility*. 1984;71(3):501-509.
13. Gundogan M, *et al.* The role of sperm surface proteins in fertilization. *Reproductive Sciences*. 2022;29(4):223-231.
14. Hess RA, Bunick D, Lee KH, *et al.* A role for estrogens in the male reproductive system. *Nature*. 1997;390(6659):509-512.
15. Hubacher D, Trussell J. A definition of modern contraceptive methods. *Contraception*. 2015;92(5):420-421.
16. Hayati A, *et al.* Oxidative stress and protein oxidation in reproductive tissues. *International Journal of Andrology*. 2010;33(4):599-610.
17. James ER, *et al.* Mechanisms of estrogen action in male reproductive health. *Endocrinology & Metabolism*. 2020;42(4):299-312.
18. Jones R. Plasma membrane modifications during mammalian sperm maturation. *International Journal of Andrology*. 1998;21(2):45-55.
19. Kiley J, Hammond C. Combined hormonal contraception: Benefits and risks. *Obstetrics and Gynecology*. 2007;110(5):1373-1380.
20. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227(5259):680-685.

21. Leridon H. Demographic perspectives on world population growth. *Population Studies*. 2020;74(1):1-16.
22. Mindnich R, Möller G, Adamski J. 17 β -Hydroxysteroid dehydrogenases and their role in androgen metabolism. *Molecular and Cellular Endocrinology*. 2004;218(1-2):7-20.
23. Naz RK, Vanek N. Role of SP-10 in sperm-egg interaction. *Molecular Reproduction and Development*. 1998;50(3):316-324.
24. Odet F, Duan C, Willis WD, *et al.* Expression of the sperm-specific LDH-C in testis and its role in sperm function. *Journal of Biological Chemistry*. 2011;286(45):40266-40275.
25. Reisse K, *et al.* Sertoli cell function and its role in spermatogenesis. *Biology of Reproduction*. 2001;65(3):567-576.
26. Shen S, Wang J, Wang Z, *et al.* Oxidative stress and its effect on sperm function. *Biochemical Journal*. 2011;437(1):35-47.
27. Sidhu A, Singh R, Sharma R. Effect of endocrine disruptors on testicular steroidogenesis. *Endocrine Research*. 2020;45(3):129-141.
28. Sitruk-Ware R, Nath A, Mishell DR. Contraception technology: Past, present, and future. *Contraception*. 2013;87(3):319-330.
29. Sullivan R, Mieusset R. The human epididymis: Its function in sperm maturation. *Human Reproduction Update*. 2016;22(3):314-329.
30. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences*. 1979;76(9):4350-4354.
31. Turner TT. The epididymis: Sperm maturation, storage, and fertility regulation. *International Journal of Andrology*. 2008;31(2):195-206.
32. Veron M, *et al.* Epididymal protein secretion and sperm function. *Cell Biology International*. 1982;6(2):89-95.
33. Wang Y, Zhang Z, Hu M, *et al.* Lactate dehydrogenase C and male infertility: A comprehensive review. *Human Reproduction Update*. 2013;19(5):521-532.
34. Wright KP, *et al.* New developments in estrogen-progestin contraceptive combinations. *Current Medical Research & Opinion*. 2020;36(4):651-660.
35. Jones WJ, Leigh JA, Mayer F, Woese CR, Wolfe RS. *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. *Archives of Microbiology*. 1983 Dec;136:254-261.