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Animal modeling methods for oligospermia: A review

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ABSTRACT

Oligospermia, characterized by a low sperm count in semen, is a major cause of male infertility and may result from various factors including infections, lifestyle choices, retrograde ejaculation, tumors, hormonal imbalances, drug treatments, and environmental exposures. Animal models play a crucial role in understanding its pathophysiology, identifying therapeutic targets, and evaluating potential treatments. The current review highlights experimental models used to induce oligospermia in laboratory animals, focusing on chemical, surgical, and radiation-based approaches. We review the reversible and irreversible methods commonly employed to study impaired spermatogenesis, along with the key endpoints used to assess testicular function and sperm quality. Standard housing and feeding conditions relevant to oligospermia research are also summarized to support reproducibility and methodological consistency in experimental designs.

KEYWORDS: Oligospermia; Infertility; Cyclophosphamide; Cisplatin; Sperm count; Sperm morphology; Testosterone; Spermatogonia

1. Introduction

It is estimated that infertility affects 8%–12% of couples globally, with a male factor being a primary or contributing cause in approximately 50% of couples[1]. An average sperm count is defined by the World Health Organization (WHO) as having at least 15 million sperm per millilitre (mL) of semen. 10 to 15 million sperm/mL is considered mild oligospermia. 5 to 10 million sperm/mL is regarded as moderate oligospermia. Sperm counts between 0 and 5 million sperm/mL are indicative of severe oligospermia[1].

The underlying cause of oligospermia determines the appropriate

course of treatment. For example, if varicocele is the cause of oligospermia, the patient may require surgical intervention to seal the swollen veins and restore normal blood flow to the testes, which can help increase sperm production and maintain a healthy testicular environment. Hormonal therapy may be required when oligospermia results from hormonal imbalances. In addition, alternative medications or adjustment of the existing drug regimen may be necessary when oligospermia is induced by certain drugs used in the treatment of other medical conditions. Various antibiotics, anticancer, antihypertensive and other medications are used to treat inflammation, and common diseases condition may causes oligospermic condition. Oligospermia can be prevented through lifestyle modifications such as maintaining a healthy diet, achieving weight loss when necessary, abstaining from tobacco products, reducing alcohol intake, and avoiding marijuana and other recreational substances, all of which may help improve sperm count[1,2].

A non-systematic literature search was performed using Google Scholar, PubMed, Scopus, and Web of Science. Keywords included “oligospermia,” “animal models of oligospermia,” “induced oligospermia,” “male infertility models,” “reproductive toxicity,” and the names of specific inducers (*e.g.*, “busulfan,” “cisplatin,” “hyperthermia,” “varicocele model”). Studies published in English between 1980 and 2024 were considered. Additional relevant

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2. Use of animal models for oligospermia

Animal models are typically used to induce experimental oligospermia because they are useful in comprehending the disease's pathophysiology. Animal models are useful for comprehending the intricate multi-systemic interconnections and complex etiology of spermatogenesis. Animals like adult male cynomolgus monkeys (*Macaca fascicularis*), guinea pigs, rabbit, rats, mice, etc. are used in many oligospermic trials. Rodents are a better fit for these kinds of studies. Animals are kept according to standard guidelines. In experimental animals, oligospermia can be induced through the exposure of chemical agents (such as anticancer, β blockers, antibiotics, etc.), surgery, radiation, oxidative-stress or thermal exposure. These models decrease spermatogenesis and cause damage to reproductive cells in the animal body, leading to a low sperm count and thereby inducing oligospermia.

3. Induction models for oligospermia

3.1. Scrotal hyperthermia-induced oligospermia

Heat is a well-established inducer of oligospermia, as elevated testicular temperature reduces sperm viability and motility, impairing mitochondrial function and adenosine triphosphate (ATP) synthesis. Spermatogenesis decreases approximately 14% for every 1 °C increase in testicular temperature, and prolonged exposure to high ambient temperatures induces oxidative stress, DNA damage, and germ cell apoptosis through mitochondrial pathways. These heat-related effects reduce sperm quality, damage testicular tissue, and increase the risk of infertility[3].

Scrotal hyperthermia is therefore widely used in rats and mice as a reproducible model of oligospermia. Hyperthermia activates caspases, heat shock proteins, and oxidative stress pathways, leading to temporary reductions in sperm count and seminiferous tubule integrity. Importantly, these effects are reversible, as testicular function begins to recover as soon as normal temperature is restored. Compared to chemotherapy or radiation models, scrotal hyperthermia offers advantages such as lower mortality, fewer systemic toxicities, and shorter recovery times[4]. This method has been effectively applied in rats[4] and mice[5] as a practical approach to induce transient oligospermia[6,7] (Table 1).

3.2. Chemically induced experimental oligospermia

Various chemicals are widely used to induce oligospermia in

experimental animals due to their ability to damage germ cells, inhibit DNA synthesis, disrupt spermatogenesis and generate oxidative stress. Among these, alkylating agents such as busulfan, cisplatin and cyclophosphamide are the most commonly employed because they cause DNA strand breaks, DNA-protein cross-links and apoptosis of rapidly dividing spermatogonia, leading to marked reductions in sperm count and long-term impairment of testicular function[8–17]. Other therapeutic drugs—including sulfasalazine, hydroxyurea, and paclitaxel—exert testicular toxicity through oxidative stress, inhibition of DNA replication, or microtubule disruption, resulting in abnormal sperm morphology, reduced motility, and impaired steroidogenesis[18–27]. Environmental toxicants such as λ -cyhalothrin, gentamicin, monosodium glutamate, and 5-fluorouracil have also been reported to decrease daily sperm production, damage seminiferous tubules, and induce germ cell apoptosis primarily through oxidative stress and genotoxicity[25–33]. In general, chemical models offer reproducibility and controlled induction of oligospermia, but the severity and reversibility of testicular damage varies depending on the agent, dose, and duration of exposure[8–34] as shown in Table 1.

3.3. Surgically induced oligospermia

In this process inducing oligospermia, some part of reproductive system is removed surgically to create abnormality in the system due to which sperm production decreases. It can be carried out by varicocele, vasectomy, ligation, and other techniques. These methods of inducing oligospermia are not recommended these days due to their irreversibility and can result in a permanent loss of fertility[35] (Table 1).

As for varicocele-induced oligospermia, the abnormal tortuosity and dilation of the pampiniform plexus veins within the spermatic cord are the hallmarks of varicocele. Although a number of reports have demonstrated the mechanisms through which varicocele impacts infertility, the precise pathophysiology of inflammation caused by varicocele and its connection to testicular endocrine disruption are still largely unclear, retrograde blood flow *via* the internal spermatic and cremasteric veins into the pampiniform plexus causes varicocele[3].

Adult male rats (450 g) were acclimatized for 1 week prior to experimental use. The oligospermia is induced surgically by left varicocele operation. 50 mg/kg intraperitoneal sodium pentobarbital was used to anesthetize rats[36]. The left renal vein was partially ligated to an external diameter of 1 mm through a midline incision, distal to the left adrenal and testicular veins. The animals' abdomen was sealed, they were put back in the vivarium, and they went through the same procedure a month later. Upon examination during the autopsy, the dilatation of the left internal spermatic vein was classified as either no dilatation,

Table 1. Summary of induction models of oligospermia with different animal and species model.

Method type	Inducer/Approach	Mechanism of action	Common animal species	Reversibility
Thermal	Scrotal hyperthermia	Heat-induced oxidative stress, mitochondrial dysfunction, germ-cell apoptosis	Rat, Mouse	Reversible
Chemical	Busulfan	Alkylation spermatogonial depletion, DNA cross-linking	Rat, Mouse	Often irreversible (dose-dependent)
Chemical	Cisplatin	Oxidative stress, DNA adducts, apoptosis of germ cells	Rat, Mouse	Partially reversible
Chemical	Cyclophosphamide	DNA alkylation and cross-linking disruption of spermatogenesis	Rat, Mouse	Dose-dependent
Chemical	Sulfasalazine	Reactive oxygen species (ROS) generation impaired steroidogenesis and sperm dysfunction	Rat	Reversible
Chemical	Hydroxyurea	Inhibition of DNA synthesis and S-phase arrest	Rat, Mouse	Reversible
Chemical	λ -Cyhalothrin	Oxidative stress and endocrine disruption	Rat, Mouse, Rabbit	Partially reversible
Chemical	Paclitaxel	Microtubule stabilization impaired germ-cell division	Rat, Mouse	Partially reversible
Chemical	Gentamicin	Oxidative stress and testicular toxicity	Rat	Reversible
Chemical	Monosodium glutamate	Oxidative stress, tubular degeneration	Rat, Mouse	Dose-dependent
Chemical	5-Fluorouracil	Antimetabolite germ-cell apoptosis, DNA damage	Rat	Often irreversible
Chemical	Doxorubicin	ROS generation germ-cell apoptosis and testicular atrophy	Rat	Mostly irreversible
Surgical	Varicocele induction	Impaired venous drainage hypoxia and elevated testicular temperature	Rat	Irreversible
Radiation	Ionizing radiation (X-ray/infrared radiation)	DNA double-strand breaks loss of spermatogonia	Mouse	Dose-dependent

moderate dilatation, or marked dilatation[37].

After the designated intervals, ranging from two to twelve weeks, the animals were sacrificed by potassium chloride injection into the left ventricle. At the time of sacrifice, the weights of the right and left testes, the seminal vesicles, and the diameter of the left spermatic vein were measured. Before the intratesticular and steroidogenic enzyme assay were performed, the testes were snap-frozen on dry ice and kept at -70°C [36,37] (Table 1).

3.4. Radiation-induced oligospermia

Short-term oligospermia can be caused by radiation; all spermatogenic cells, including differentiating spermatogonia, are most susceptible to radiation-induced death. They are dividing quickly, which is largely the cause of this sensitivity. Spermatocytes and spermatids are less vulnerable to these agents because they only need to go through one round of DNA synthesis and two meiotic divisions. Even if they have damage to their chromosomes and DNA, they can still proceed through their differentiation sequence and develop into sperm. Three weeks are typically observed as a lag between the initiation of treatment and the main decline in sperm counts, based on the kinetics of spermatogenesis[38].

Oligospermia may persist as long as an animal receives radiation that destroys differentiating spermatogonia. Although stem cells live, as soon as they cross that delicate stage, their offspring are eliminated. In 6 to 8 weeks in mice and 12 weeks in rats, sperm

production returns to normal following treatment termination, assuming minimal stem spermatogonia killing occurs[38].

Male mice, aged 1.5 months and of average weight 28 g, were each given an intraperitoneal injection of $1.2\ \mu\text{C}$ adenine-8- ^{14}C (specific activity $4.6\ \text{mc/mM}$) per gram of body weight. Three days later, three males were irradiated with 200 r of X-rays to the testes while under Nembutal anaesthesia. The constants of the X-ray machine were: 140 kVp, 220 r/minute, inherent filtration $\approx 1\ \text{mm Al.}$, plus $1\ \text{mm Al.}$ Filter, H.V.L. $\approx 3.2\ \text{mm Al}$ [39] (Table 1).

4. Evaluation parameters

Evaluation of experimental models of oligospermia typically focuses on sperm quality, markers of oxidative stress, hormonal status, DNA integrity, and testicular histopathology. These parameters collectively indicate the degree of spermatogenic impairment induced by different experimental approaches.

4.1. Characteristics of epididymal sperm

The epididymis plays a critical role in sperm maturation, acquisition of motility and fertilization capacity[5]. Assessment of spermatozoa obtained from the cauda epididymis includes sperm count, viability, motility, and morphology, which are essential indices of impaired spermatogenesis.

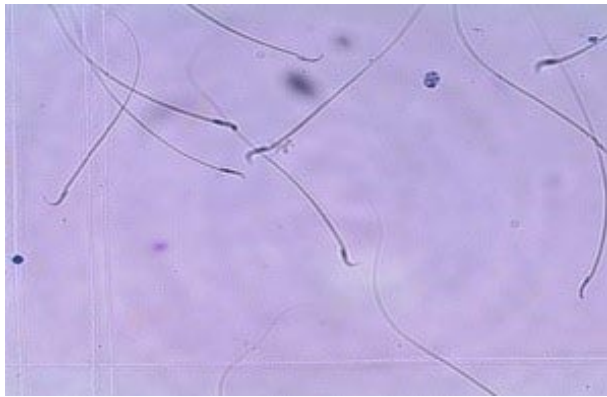


Figure 1. Rat sperms observed under microscope. Representative image shows sperm cells obtained from rat semen and observed under a light microscope at 40× magnification. The image illustrates the typical morphology of rat spermatozoa, including the distinct oval-shaped head, short neck (midpiece), and long, slender flagellum (tail) responsible for motility.

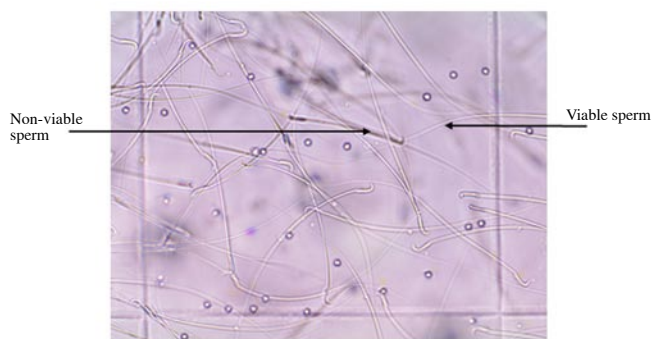


Figure 2. Microscopic visualization of rat sperms following trypan blue staining. Representative image of rat sperm cells observed under a light microscope at 40× magnification after trypan blue staining, a dye commonly used to assess sperm viability. In this staining method, non-viable (dead) sperm cells absorb trypan blue and appear stained, whereas viable (live) sperm cells exclude the dye and remain unstained or lightly colored.

4.1.1. Sperm count

A sperm concentration below 15 million/mL is indicative of oligospermia[40]. Reduced sperm count directly reflects damage to spermatogenic cells and testicular function[41,42] as indicated in Figure 1.

4.1.2. Sperm viability

Viability evaluates the proportion of sperm with an intact membrane (alive) and is an important indicator, especially when motility is poor[41,42]. Viable sperm remain colourless while non-viable sperm absorb vital dyes as nigrosine, eosin Y, trypan blue[26] shown in Figure 2.

4.1.3. Sperm motility

Motility is essential for fertilization and is often compromised in oligospermia due to mitochondrial dysfunction or structural abnormalities[43]. The percentages of motile and nonmotile sperm are typically quantified microscopically[43].

4.1.4. Sperm morphology

Abnormalities such as defects in the head, midpiece, or tail indicate disturbances in spermiogenesis[44] as shown in Figure 3. Morphological assessment helps identify structural changes induced by toxic substances or stress[45].

4.2. Biochemical tests

Biochemical markers provide information about oxidative stress and steroidogenic function, two critical pathways disrupted in oligospermia.

4.2.1. Catalase activity

Catalase degrades hydrogen peroxide and serves as an important antioxidant enzyme in testicular tissue[46-51]. Reduced catalase activity suggests elevated oxidative stress, a common mechanism in chemically or thermally induced oligospermia.

4.2.2. Reduced glutathione (GSH)

GSH protects germ cells from reactive oxygen and nitrogen species[51,52]. Its quantification using the 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent reflects the antioxidant capacity, with decreased levels indicating oxidative imbalance[53].

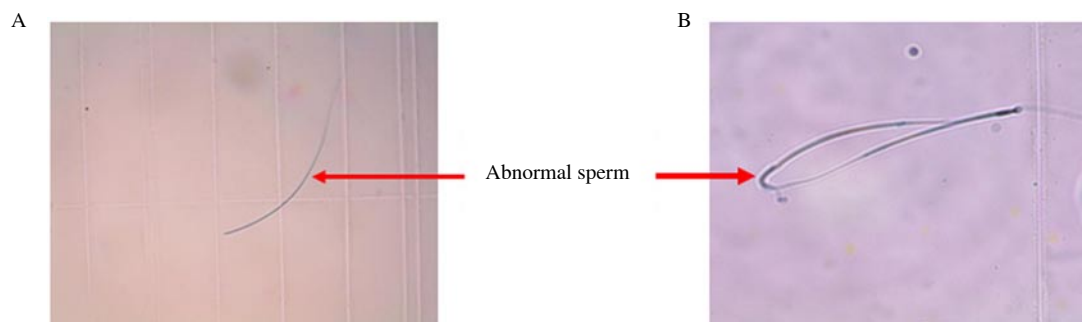


Figure 3. Microscopic observation of abnormal rat sperm. Representative images (A, B) show abnormal sperm cells collected from rat semen and observed under a light microscope at 40× magnification. The images illustrate several sperms displaying morphological abnormalities, including irregularly shaped heads, bent or coiled tails, and structural deformities of the midpiece.

4.2.3. 3β -hydroxysteroid dehydrogenase (3β -HSD) and 17β -hydroxysteroid dehydrogenase (17β -HSD) enzymes

These enzymes (3β -HSD and 17β -HSD) regulate testosterone biosynthesis and are crucial for normal spermatogenesis[54–56]. Changes in its activity indicate impairment of steroidogenesis, commonly observed in testicular dysfunction induced by toxic substances.

4.3. Serum hormone levels

Sex hormones—including testosterone, estradiol, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and sex hormone-binding globulin (SHBG) play essential roles in spermatogenesis[57,58]. The hypothalamic-pituitary-gonadal axis regulates germ cell development, and disruption of LH/FSH secretion or testosterone production leads to reduced sperm production[59,60]. The hormonal profile therefore provides supporting evidence for the induction of oligospermia.

4.4. DNA fragmentation and cell death

Oligospermia is often accompanied by increased germ cell apoptosis or DNA damage.

4.4.1. Sperm chromatin dispersion (SCD) test

The SCD test identifies sperm with fragmented DNA based on the presence or absence of characteristic halos[61,62].

4.4.2. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) test

The TUNEL method detects apoptotic cells by marking DNA strand breaks and is widely used to evaluate testicular apoptosis in oligospermia models[63].

4.4.3. Comet assay

This single-cell gel electrophoresis method measures DNA strand breaks and provides quantitative assessment of genomic integrity[64].

4.5. Histopathological analysis

Histological evaluation of hematoxylin and eosin (H & E)-stained testicular sections reveals changes in the seminiferous tubules, including reduced epithelial height, smaller tubular diameter, and decreased numbers of spermatogonia, spermatocytes, and spermatids[65,66]. These changes directly reflect impaired spermatogenesis in models of induced oligospermia.

5. Study's limitations

The experimental models used to investigate oligospermia have

several inherent limitations. Many of these models do not fully recapitulate human physiological or pathological conditions, reducing the translational relevance of the findings to clinical practice. Considerable heterogeneity exists across studies due to differences in animal species, strains, induction methods (chemical, thermal, surgical, or radiation-based), and evaluation parameters, making comparisons difficult. Furthermore, most studies focus on short-term outcomes and often omit long-term assessments of fertility or reproductive toxicity. Adverse effects and systemic toxicity of experimental interventions may also be underreported, limiting accurate safety assessment. Furthermore, variations in semen analysis protocols, hormonal testing, and histopathological grading contribute to inconsistencies that may affect interpretation of treatment efficacy.

6. Conclusions and future prospects

Animal models play an important role in furthering the understanding of oligospermia, a major cause of male infertility due to decreased sperm count. This review highlights commonly employed experimental approaches—including chemical, thermal, surgical, and radiation-induced models—that generate both reversible and irreversible forms of oligospermia. These models provide valuable platforms to elucidate the mechanisms underlying impaired spermatogenesis and evaluate potential therapeutic interventions.

Looking ahead, more attention is needed to strengthen the translational relevance of oligospermia research in many fields. Refinement of existing models is needed to better mimic the diverse etiologies and complexities of human oligospermia. Integrating patient-specific genetic, environmental, and epigenetic factors into experimental designs may aid progress toward personalized therapeutic strategies. Expanding research on non-hormonal treatments may offer alternative treatment avenues with fewer side effects than traditional hormonal approaches. Additionally, deeper investigation of the roles of environmental exposures and epigenetic modifications may help elucidate disease mechanisms and inform preventive strategies.

By addressing these future directions, the use of animal models may significantly increase our understanding of oligospermia and contribute to the development of more effective and personalized treatment approaches for male infertility.

Conflict of interest statement

The authors declare that they have no competing interests regarding the publication of the data included in the manuscript.

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Authors' contributions

Dinesh Sharma helped in conceptualization of the review and data compilation. Nitesh Parsaila contributed to literature search and initial draft writing. Archana Navale played a crucial role in supervision, data analysis and critical revision of the manuscript. Sagar Shinde reviewed experimental models and pharmacological interventions and contributed to the methodology section. Nishant Patidar and Prachi Nahar assisted in manuscript structuring, reference management, and proofreading. Ajay Shelke contributed to critical evaluation of pharmacological agents and editing the discussion section. Nilay Solanki helped in refining the introduction and conclusion and was involved in final review and approval of the manuscript.

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