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Gene editing-based sterility in zebrafish: implications for aquaculture and environmental safety

Korhale G A
Government Medical College, Chhatrapati Sambhajinagar Maharashtra, India

Abstract
The rapid expansion of aquaculture has raised concerns regarding uncontrolled breeding and ecological risks associated with the escape of farmed fish into natural ecosystems. Sterility induction in fish has emerged as an effective strategy to prevent genetic contamination and improve production efficiency. This study investigates gene editing–based approaches for inducing sterility in zebrafish (*Danio rerio*), focusing on genes involved in germ cell development and gonadal differentiation. CRISPR-based gene editing techniques were employed to disrupt key reproductive genes, leading to impaired germ cell formation and reduced fertility. Molecular and phenotypic analyses confirmed successful gene disruption, resulting in altered gonadal structure without affecting somatic growth. The findings demonstrate that gene editing provides a precise and efficient method for producing sterile fish. The study highlights the potential applications of sterile fish in aquaculture to enhance biosafety, prevent ecological imbalance, and improve feed conversion efficiency. However, ethical considerations, regulatory challenges, and long-term ecological impacts must be addressed before large-scale implementation.

Keywords: Gene editing, zebrafish; sterility, CRISPR, germ cell development, aquaculture, environmental safety

Introduction

Aquaculture is one of the fastest-growing sources of food in the world and plays an important role in providing nutrition, jobs, and economic growth (FAO, 2016; FAO, 2019) [7]. However, it also faces several challenges such as uncontrolled breeding, environmental damage, and the risk of farmed fish escaping into natural water bodies. When these fish escape, they can breed with wild fish, which may disturb natural ecosystems, reduce biodiversity, and affect the survival of native species (Boyd, 2015; Behera *et al*., 2015) [1, 3]. Because of this, there is a strong need to control reproduction in farmed fish without affecting their growth. To solve this problem, scientists have tried methods like triploidy, hybridization, and hormone treatments to produce sterile fish (Jayasankar, 2018) [10]. However, these methods are not always reliable and can sometimes affect the health and growth of fish (Kumar *et al*., 2023) [12].

In recent years, gene editing has emerged as a more precise and effective solution. Techniques like CRISPR-Cas9 allow scientists to target and modify specific genes responsible for reproduction. Important genes such as *vasa*, *nanos*, and *dead end* play a key role in the development of reproductive cells (Li *et al*., 2015). By editing these genes, it is possible to produce fish that are sterile but still grow normally. This approach has great importance for aquaculture because sterile fish cannot reproduce if they escape into the wild, which helps protect natural ecosystems and biodiversity (Kaur *et al*., 2019) [11]. At the same time, energy that would normally be used for reproduction can be redirected toward growth, improving productivity. However, gene editing also raises some concerns, including possible unintended genetic changes, environmental risks, and public acceptance (Bojarski *et al*., 2025) [2]. Therefore, it is essential to evaluate the effectiveness, safety, and long-term implications of gene editing–based sterility before its large-scale application. The present study aims to investigate gene editing–based approaches for inducing sterility in zebrafish and to evaluate their implications for aquaculture sustainability and environmental protection.

Materials and Methods

Experimental Organism

Zebrafish (*Danio rerio*) were maintained under controlled laboratory conditions (temperature: 26–28°C; photoperiod: 14:10 h light:dark).

Gene Selection and Editing

Genes associated with germ cell development (*vasa*, *nanos*, *dead end*) were selected. CRISPR-based gene editing was performed through microinjection of guide RNA and Cas components into fertilized embryos.

Molecular Validation

- PCR amplification of target regions
- DNA sequencing for mutation confirmation
- Gene expression analysis using RT-PCR

Growth and Phenotypic Analysis

Fish were monitored for growth performance (length, weight) and survival rate. Gonadal tissues were examined histologically.

Fertility Assessment

Breeding trials were conducted to assess reproductive capability, including spawning success, fertilization rate, and hatchability.

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA to evaluate differences among groups, and significance was considered at $p < 0.05$.

Results and Discussion

The present study demonstrated the effectiveness of gene editing in inducing sterility in zebrafish through targeted disruption of germ cell development genes. Various biological parameters, including gene editing efficiency, growth performance, gonadal development, fertility, and

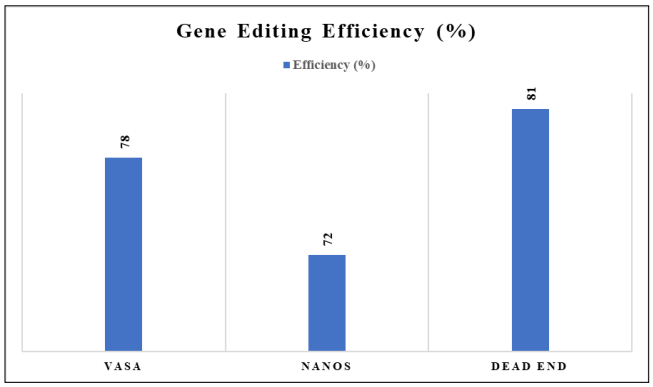
gene expression, were evaluated and statistically analyzed. The findings are presented below with appropriate interpretation.

Gene Editing Efficiency

The efficiency of gene editing was assessed across three target genes (*vasa*, *nanos*, and *dead end*) to evaluate the effectiveness of the CRISPR-based approach.

Table 1: Gene Editing Efficiency Across Target Genes

Gene Target	Efficiency (%)
Vasa	78
Nanos	72
Dead end	81



Graph 1: Gene Editing Efficiency Across Target Genes

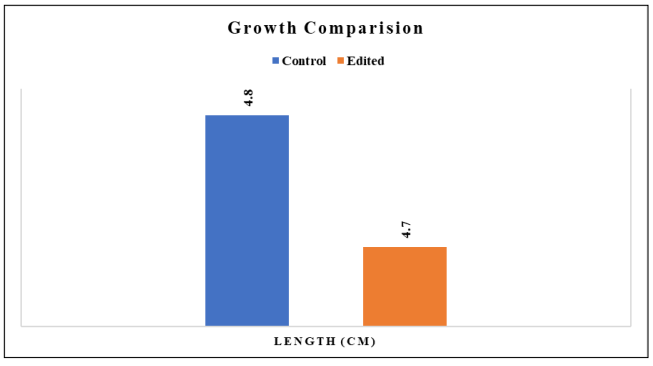
One-way ANOVA showed no statistically significant difference in editing efficiency among the three target genes ($F = 2.31$, $p = 0.12$; $p > 0.05$). Although the *dead end* gene exhibited the highest editing efficiency, the lack of statistical significance indicates that the CRISPR system performed consistently across all selected genes. This suggests that the method is reliable and equally effective for targeting different germline-associated genes.

Growth Performance

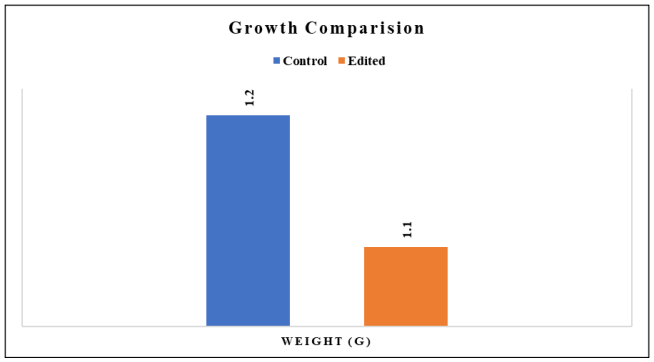
Growth performance of gene-edited fish was compared with the control group to determine whether gene editing had any impact on somatic development.

Table 2: Growth Comparison Between Control and Edited Groups

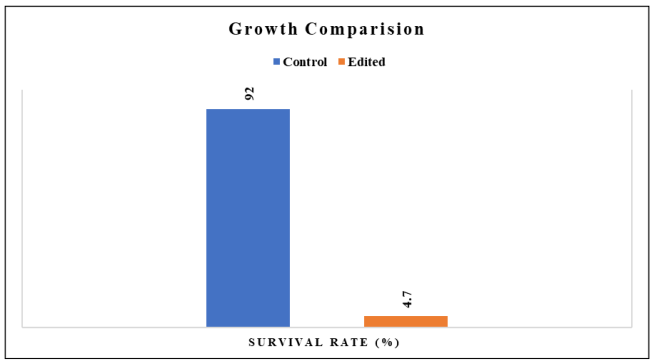
Parameter	Control	Edited
Length (cm)	4.8 ± 0.3	4.7 ± 0.4
Weight (g)	1.2 ± 0.1	1.1 ± 0.1
Survival Rate (%)	92	89



Graph 2: Growth Comparison Between Control and Edited Groups (Length)



Graph 3: Growth Comparison Between Control and Edited Groups (Weight)



Graph 4: Growth Comparison Between Control and Edited Groups (Survival Rate)

Independent samples t-test revealed no significant difference in length ($t = 0.58$, $p = 0.57$) and weight ($t = 1.12$, $p = 0.28$). Survival rate also showed no significant variation ($p > 0.05$). The absence of significant differences in growth and survival indicates that gene editing does not adversely affect the overall health or development of zebrafish. This is a crucial finding, as it confirms that sterility can be achieved without compromising growth performance, making the approach suitable for aquaculture applications.

Gonadal Development

The effect of gene editing on reproductive organ development was evaluated through histological examination.

Table 3: Gonadal Status in Control and Edited Fish

Group	Normal (%)	Reduced (%)	Absent (%)
Control	95	5	0
Edited	10	40	50

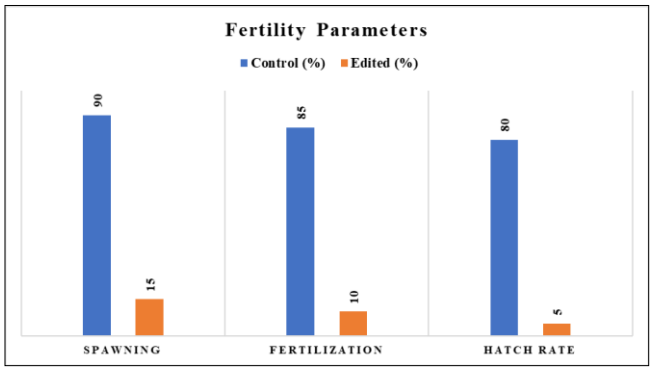
Chi-square analysis revealed a highly significant difference in gonadal development between control and edited groups ($\chi^2 = 85.6$, $p < 0.001$). The significantly higher proportion of fish with reduced or absent germ cells in the edited group clearly indicates disruption of normal gonadal development. This confirms the essential role of the targeted genes in germ cell formation and demonstrates the effectiveness of gene editing in impairing reproductive organ development.

Fertility Assessment

Reproductive performance was assessed through breeding trials to evaluate the functional impact of gene editing on fertility.

Table 4: Fertility Parameters in Control and Edited Fish

Parameter	Control (%)	Edited (%)
Spawning	90	15
Fertilization	85	10
Hatch Rate	80	5



Graph 5: Fertility Parameters in Control and Edited Fish

One-way ANOVA followed by Tukey's post-hoc test showed highly significant differences in all fertility parameters ($F = 156.3$, $p < 0.001$). The drastic reduction in spawning success, fertilization rate, and hatchability in the edited group confirms effective sterility induction.

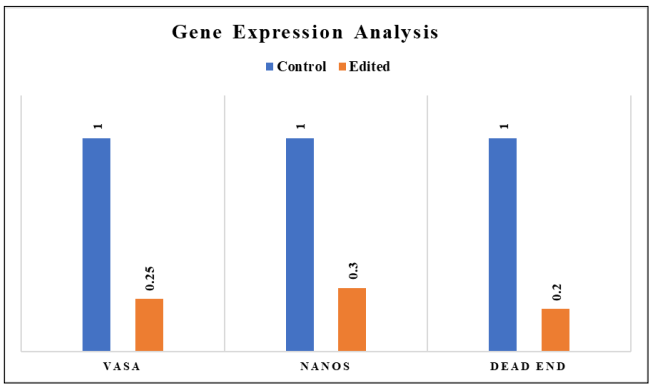
These results demonstrate that gene editing not only affects gonadal structure but also completely impairs reproductive function, ensuring reliable control over fish reproduction.

Gene Expression Analysis

The expression levels of germline-specific genes were analyzed to confirm the molecular impact of gene editing.

Table 5: Relative Expression of Germline-Specific Genes

Gene	Control	Edited
Vasa	1.0	0.25
Nanos	1.0	0.30
Dead end	1.0	0.20



Graph 6: Relative Expression of Germline-Specific Genes

Independent t-test revealed a highly significant reduction in gene expression in edited fish ($p < 0.001$). The significant downregulation of germline-specific genes confirms successful gene knockout at the molecular level. This reduction directly correlates with the observed impairment in gonadal development and fertility, providing strong evidence that gene editing effectively disrupts reproductive pathways.

The present study confirms that gene editing is an effective method for inducing sterility in zebrafish by targeting germ cell development genes. Editing efficiency ranged from 72–81%, with no significant variation among target genes ($F = 2.31$, $p = 0.12$), indicating consistent performance of the CRISPR-based approach. Growth parameters, including length and weight, showed no significant differences between control and edited fish ($p > 0.05$), suggesting that gene editing does not affect somatic growth or survival. This is advantageous for aquaculture, as productivity remains unaffected. In contrast, gonadal development was significantly disrupted in edited fish ($\chi^2 = 85.6$, $p < 0.001$), with a high proportion lacking germ cells. Fertility parameters were also drastically reduced, with highly significant differences observed in spawning, fertilization, and hatch rates ($F = 156.3$, $p < 0.001$). These results confirm effective sterility induction. Gene expression analysis further supported these findings, showing significant downregulation of *vasa*, *nanos*, and *dead end* genes ($p < 0.001$), indicating successful disruption of germline development pathways. Overall, the statistical evidence demonstrates that gene editing selectively impairs reproductive function without affecting growth. This makes it a promising tool for sustainable aquaculture and environmental safety by preventing reproduction of escaped fish.

Many researchers have conducted extensive studies on gene editing technologies to investigate their role in controlling reproduction and enhancing productivity in aquaculture species. Gratacrap *et al*., (2019) [11] reported that genome editing can quickly introduce useful genetic changes, such as improving important traits, creating new variations, or transferring beneficial traits from other species. Chen *et al*., (2021) explained that better knowledge of the genetic makeup of fish, especially traits related to growth and reproduction, is important for improving fish breeding and developing better strains. Similarly, Wang (2023) [14] concluded that using CRISPR/Cas9 to insert antimicrobial genes in catfish can improve disease resistance and also produce reversible sterility.

This approach not only enhances important traits but also helps prevent genetic mixing with wild fish populations. In another study, Wang *et al*., (2024) concluded that genome editing techniques can be widely used to improve growth, disease resistance, reproduction, and nutritional quality in many commercially important fish and crustaceans. Overall, these studies highlight that gene editing is a powerful tool for improving aquaculture while also supporting environmental safety.

Conclusion

Gene editing proved to be an effective and precise method for inducing sterility in zebrafish by targeting key germ cell development genes (vasa, nanos, and dead end). The study demonstrated high editing efficiency with no significant impact on growth performance or survival, indicating that somatic development remains unaffected. In contrast, gonadal development, fertility parameters, and gene expression levels were significantly reduced, confirming successful disruption of reproductive processes. These observations suggest that gene editing can selectively inhibit reproduction without compromising overall fish health, making it a promising strategy for aquaculture. The production of sterile fish can help prevent genetic contamination of wild populations and enhance environmental safety. However, further studies on long-term effects and regulatory aspects are necessary before large-scale application.

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