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A Review of Oocyte Maturation and Ovulation Mechanisms in Zebrafish: Role of DHP, DES, and Gene Editing

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ABSTRACT-

Infertility affects approximately 20% of couples worldwide, making zebrafish research increasingly valuable for understanding reproductive mechanisms. Recent breakthroughs in this field have significantly advanced our knowledge of fertility control and ovarian development. Zebrafish has emerged as an excellent model system to study the control of ovarian development, allowing scientists to investigate dozens of genes involved in reproduction through targeted gene knockout techniques. Furthermore, our understanding of zebrafish reproduction has expanded with discoveries about hormonal triggers. Specifically, researchers found that diethylstilbestrol (DES) triggers oocyte maturation within several hours when administered directly into surrounding water . The implementation of a controllable sterility strategy is also crucial for commercializing precise trait improvements in farmed fish using genome editing . In this article, we explore how the administration of exogenous 17α,20β-dihydroxy-4-pregnen-3-one (DHP) at specific concentrations effectively restores fertility in genetically modified female zebrafish , creating potential applications for sustainable aquaculture and reproductive medicine.

Keywords: Zebrafish, Female Fertility Control, Oocyte Maturation, Diethylstilbestrol (DES), 17α,20β-Dihydroxy-4-Pregnen-3-One (DHP) etc.

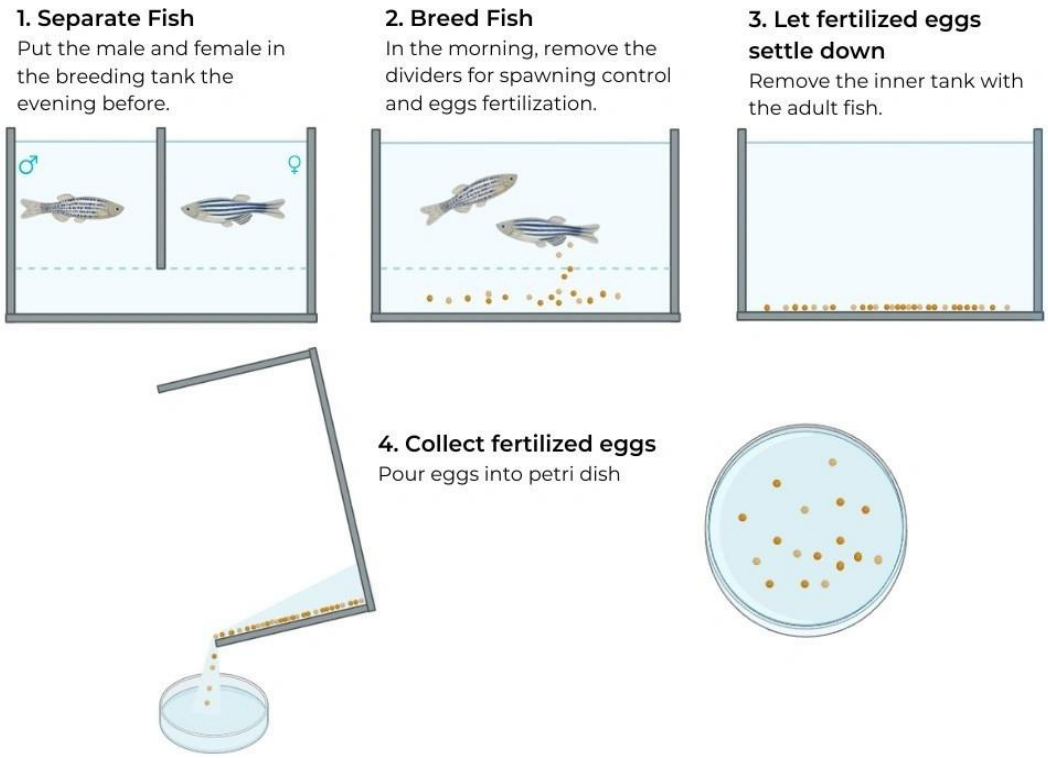
Researchers identify DHP as key to restoring zebrafish ovulation

Scientists have discovered that 17α,20β-dihydroxy-4-pregnen-3-one (DHP) plays a critical role in zebrafish reproduction through dual action pathways. This maturation-inducing hormone (MIH) operates via both non-genomic and genomic mechanisms to coordinate the complete reproductive cycle [1].

DHP triggers oocyte maturation in lhβ- and star-deficient females

Recent zebrafish research reveals that DHP effectively restores reproductive function in genetically modified females with fertility issues. In a groundbreaking study, researchers administered DHP at concentrations of 100 and 300 µg/L for 6 hours (from 02:00 to 08:00 a.m.) to lhβ- and star-deficient females respectively [2]. These mutant females normally cannot produce mature eggs due to disruptions in their reproductive hormone pathways.
The transformation begins rapidly after DHP exposure. In lhβ-deficient females, researchers observed that non- transparent follicles began transitioning to semi-transparent after just 2 hours of DHP treatment, indicating the initiation of oocyte maturation [2]. Moreover, after 4 hours of exposure to 100 µg/L DHP, anatomical examination revealed transparent follicles embedded in the dissected ovaries with some eggs forming membranes - a characteristic of stage V maturation [2].

Interestingly, the required DHP concentration differs between mutant types. While 100 µg/L sufficiently rescued oocyte maturation in lhβ-deficient females, star-deficient females required a higher concentration of 300 µg/L to achieve similar results [2]. This difference highlights the varying degrees of hormonal disruption in different genetic mutations.



Fertility restored through short-term hormone exposure

The time course of DHP-induced fertility restoration follows a predictable pattern. In zebrafish, oocyte maturation normally occurs within three hours of DHP exposure, with ovulation following within four hours in vivo [1]. This timeline proved consistent in the mutant recovery experiments as well.

Following the 6-hour DHP treatment protocol, researchers placed the treated lhβ-deficient females in breeding tanks with wild-type males. The results were remarkable - 76.00 ± 23.32% of DHP-treated lhβ-deficient females successfully spawned [2]. Of these eggs, 25.90 ± 12.28% were fertilized, 68.11 ± 31.45% of fertilized eggs broke their membrane, and 59.80 ± 39.56% survived to juvenile stage [2].

For star-deficient females, the higher concentration treatment (300 µg/L) yielded similarly promising results, with 51.90 ± 18.84% of treated females spawning [2]. The fertilization rate was notably higher than in lhβ-deficient females, reaching 72.87 ± 9.92% [2]. Additionally, 47.81 ± 26.25% of fertilized eggs broke their membrane, and 66.04 ± 32.40% of these developed to the juvenile stage [2].

In contrast to other hormonal treatments, DHP uniquely addresses both maturation and ovulation. Whereas DES induces only oocyte maturation but not ovulation [3], DHP triggers both processes. This dual action results from DHP binding to both membrane-bound progestin receptors (mPRs) for maturation and nuclear progesterone receptors (nPRs) for ovulation [1].

Consequently, these findings establish DHP as a potent fertility switch in genetically modified zebrafish, offering new possibilities for controlled reproduction in research and aquaculture applications.

Scientists confirm DES mimics non-genomic steroid action

Diethylstilbestrol (DES), a non-steroidal estrogen and endocrine-disrupting chemical, has become instrumental in uncovering critical aspects of steroid action in zebrafish reproduction. Through extensive research, scientists have established that DES effectively mimics the non-genomic actions of progestins, providing valuable insights into how steroids influence oocyte development without genomic involvement [4].

DES induces oocyte maturation but not ovulation

Research reveals that DES triggers oocyte maturation in zebrafish when administered directly into surrounding water, with maturation occurring within several hours [5]. This process closely resembles physiological maturation as judged by multiple criteria, including morphological changes [6]. During this transformation, oocytes become transparent – a characteristic feature of matured eggs [6].

However, a critical distinction exists between DES and natural progestins: DES induces oocyte maturation but fails to trigger ovulation, even after extended incubation periods [5]. This selective action contrasts sharply with progestins such as progesterone, 17α-OH progesterone, and 17,20β-DHP, which successfully induce both maturation and ovulation [5].

The time-course of oocyte maturation induced by DES parallels that triggered by 17,20β-DHP, suggesting both compounds activate similar initial pathways [6]. This finding indicates that DES primarily targets early maturation mechanisms rather than the complete reproductive sequence. Indeed, the process of meiosis resumption during final oocyte maturation (FOM) serves as an excellent model for studying non-genomic steroid actions in zebrafish [1].

Essentially, DES-induced maturation follows the same non-genomic signaling cascade as natural progestins: binding to a membrane receptor initiates a series of rapid cellular responses that occur at the oocyte surface without requiring nuclear involvement or transcription [1]. These responses include down-regulation of cAMP and up-regulation of MAPK signaling pathways [3].

DES binds to membrane progestin receptor (mPRα)

Direct binding studies confirm that DES interacts with membrane progestin receptor alpha (mPRα) [5]. This interaction was demonstrated through competitive binding assays using membrane fractions from cells transfected with goldfish mPRα cDNA. In these experiments, DES effectively competed with isotope-labeled 17,20β-DHP for binding to mPRα [5].

The identity of mPRα as a ~40-kDa protein was confirmed through Western blot analysis using antibodies against an N- terminal fragment predicted to be an external cellular region [4]. This antibody cross-reacted with protein extracts from both goldfish and zebrafish immature oocytes [6].

Remarkably, antibodies targeting mPRα blocked both 17,20β-DHP-induced and DES-induced oocyte maturation [7]. This inhibition strongly suggests that both compounds act as agonists for mPRα, with DES effectively mimicking the natural progestin's action [6].

Further evidence comes from studies showing the oocyte maturation-inducing activity of DES correlates directly with its agonistic activity on mPRα [8]. The binding of these substances to mPRα initiates the cascade of non-genomic progestin signaling leading to meiosis resumption [1].

Unlike nuclear progesterone receptor (Pgr), which is restricted to follicular cells and absent in oocytes, mPRα is present in the oocyte membrane where it mediates these rapid non-genomic responses [1]. Studies with Pgr-knockout zebrafish decisively demonstrated that while Pgr is essential for genomic signaling and ovulation, it is not required for the non-genomic signaling that leads to final oocyte maturation [1].

This research conclusively establishes that DES can effectively mimic natural progestins' non-genomic actions by binding to mPRα [5], providing valuable insights into steroid receptor mechanisms and potential targets for endocrine- disrupting chemicals in zebrafish reproduction.

Study reveals ovulation requires genomic signaling via Pgr

Recent genetic studies have uncovered a critical distinction between oocyte maturation and ovulation in zebrafish reproduction. While both processes occur in sequence during normal reproduction, research demonstrates they operate through separate molecular pathways that require different signaling mechanisms.

pgr-deficient zebrafish fail to ovulate despite maturation

Genetic studies with nuclear progesterone receptor (Pgr) knockout zebrafish have provided decisive evidence about ovulation mechanisms. Homozygous Pgr-knockout females are completely infertile despite having normal oocyte development [1]. This infertility stems from a specific defect: although oocytes mature normally from stage IV to stage V, they remain trapped within follicular cells, unable to ovulate [1].

Particularly revealing is that male Pgr-knockout zebrafish maintain normal fertility [1], indicating the receptor's sex- specific role in reproduction. Anatomical examination of Pgr-deficient females shows a significant increase in ovary size and gonadosomatic index compared to wild-type fish [9]. This enlargement occurs because mature oocytes accumulate within the ovary instead of being released.

Microscopic examination of surgically removed ovaries from Pgr-knockout fish reveals fully mature stage V oocytes trapped within follicular cells [3]. In contrast, wild-type females successfully release these mature oocytes during normal spawning [3]. The observation of stage V mature oocytes within Pgr-knockout ovaries confirms that final oocyte maturation proceeds normally, yet ovulation fails to occur.

DHP fails to rescue ovulation in absence of Pgr

The role of Pgr becomes even clearer through rescue experiments. Remarkably, neither human chorionic gonadotropin (HCG) nor DHP treatments can rescue ovulation in Pgr-deficient females [10], although both typically induce ovulation in wild-type fish. Histological analysis demonstrates that the outer layer cells of mature follicles fail to break down in Pgr-deficient fish, preventing egg release [11].

DHP administration at various concentrations proved ineffective at restoring ovulation in Pgr-knockout females [11], conclusively demonstrating that Pgr signaling is indispensable for DHP-mediated ovulation in zebrafish [11]. Nevertheless, DHP-induced oocyte maturation remained unimpaired in these same fish [2], confirming that maturation and ovulation rely on separate pathways.

At the molecular level, Pgr functions as an upstream regulator for critical ovulation-related genes. The expression of several metalloproteinases essential for follicle rupture is drastically reduced in Pgr-knockout fish:

- *adam8b* (a disintegrin and metalloprotease)
- *adamts9* (a disintegrin-like and metalloproteinase with thrombospondin motifs)
- *mmp9* (matrix metalloproteinase 9)

These enzymes normally increase expression in preovulatory follicles, primarily under Pgr regulation [2]. Although DHP triggers the expression of *adamts9* in wild-type fish, this response is completely blocked in Pgr-knockout fish [2], illustrating how progestins require Pgr to upregulate the proteolytic enzymes necessary for follicle rupture.

The role of Pgr in ovulation appears highly conserved across vertebrates, as evidenced by similar anovulation phenotypes in Pgr-knockout rats and mice [12]. This conservation underscores the fundamental importance of genomic progestin signaling via Pgr for successful ovulation across species, despite the diversity of reproductive strategies.

Gene markers adamts9 and adam8b linked to ovulation success

Molecular analysis has identified two key metalloproteinases—adamts9 and adam8b—as critical markers for successful ovulation in zebrafish research models. These enzymes function as proteolytic agents that facilitate follicle rupture,
enabling mature eggs to be released from the ovary.

Expression increases with DHP treatment

Extensive molecular studies reveal that DHP administration effectively upregulates the expression of both adamts9 and adam8b genes in pre-ovulatory follicle cells of wild-type females [11]. This upregulation precisely mimics what occurs during natural mating with wild-type males. Interestingly, the expression of these genes rises dramatically just before ovulation occurs, with peak levels detected approximately one hour before light exposure [11].
DHP exposure triggers a rapid molecular response in follicular cells. Within just two hours of treatment (from 05:00 to 07:00), wild-type females exhibit significantly elevated expression of both *adamts9* and *adam8b* compared to untreated controls [11]. At the cellular level, this expression is restricted specifically to granulosa cells surrounding the oocyte, with no detectable expression in adjacent oocyte or theca cells [13].
The specificity of this response is particularly revealing. Unlike *adamts9* and *adam8b*, other metalloproteinases such as *adamts1* and *mmp9* do not show similar upregulation patterns following DHP treatment [11]. This selective activation suggests these two genes serve as specialized molecular components in the ovulation process.

Markers absent in untreated mutants

In striking contrast, the increased expression of *adamts9* and *adam8b* is completely absent in untreated *lhβ*- or *star*- deficient females, even when paired with wild-type males [11]. Given that these mutants cannot ovulate naturally, this absence strongly correlates with their infertility.
The importance of *adamts9* becomes especially evident through knockout studies. From 1,047 fish generated by crossing *adamts9+/-* pairs, researchers found significantly fewer adult *adamts9-/-* fish (only 4%) than the 25% predicted by Mendelian genetics [14]. Even more telling was the dramatic gender imbalance—82% of the surviving mutants were male, while only 7% were female [14]. The remaining 11% had abnormal gonadal structures, featuring transparent, ovarian-like membranous shells rather than normal reproductive organs [14].
None of the female *adamts9* knockouts could release eggs, regardless of circumstances [14]. Correspondingly, histological examination confirmed no ovulated oocytes were present in these mutants [14]. Given these findings, researchers concluded that arrested expression of *adamts9* and *adam8b* represents one of the major reasons for failed oocyte maturation and ovulation in zebrafish reproduction [11].
The ability of DHP to restore expression of these critical genes explains its effectiveness as a fertility switch. Even in previously non-expressing *lhβ*- or *star*-deficient females, a brief 2-hour DHP exposure successfully upregulates both *adamts9* and *adam8b* [11], initiating the molecular cascade required for successful ovulation.

Breakthrough enables fertility control in genome-edited fish

The practical application of DHP-induced fertility control opens new horizons for aquaculture innovation, offering solutions to long-standing challenges in fish farming practices.

DHP enables 'off-on' fertility switch in aquaculture

The administration of exogenous DHP at carefully calibrated concentrations serves as an effective "off-on" switch for managing fertility in genome-edited cyprinids [11]. This breakthrough builds upon recent success in generating all- female carp populations through *cyp17a1*-depletion, creating a versatile fertility control system [15]. What makes this approach revolutionary is its temporary nature—fertility can be suppressed until desired, then restored via brief hormone exposure.
Tests demonstrate that fertilized eggs from DHP-treated mutant females develop normally for up to three weeks post- fertilization [15], confirming the viability of offspring produced through this method. Hence, producers can maintain sterile populations for commercial purposes yet restore fertility whenever breeding stock requires replenishment.

Potential for eco-friendly breeding strategies

Beyond laboratory applications, this fertility control technology addresses critical environmental concerns in modern aquaculture. Farming infertile fish represents the most effective genetic-containment strategy for environmentally- responsible fish farming [6].

Accordingly, controlled sterility prevents ecological disruption caused by escaped farmed fish, eliminating risks of genetic contamination to wild populations [6].

Additional benefits exist alongside environmental protection. Sterility enhances muscle development by redirecting energy typically used for gonadal growth [6]. This approach simultaneously prevents sexual maturation that would otherwise cause deterioration of flesh quality [6], thus increasing yield quality throughout production cycles.

Conclusion

The remarkable progress in zebrafish research has significantly advanced our understanding of female fertility control mechanisms. Through careful genetic manipulation and hormone studies, scientists have unraveled the distinct pathways governing oocyte maturation and ovulation. Undoubtedly, the discovery that DHP functions as an effective fertility switch represents a major breakthrough, particularly for genetically modified fish with reproductive deficiencies.

DHP stands out among hormonal treatments because it addresses both maturation and ovulation processes. While DES triggers only oocyte maturation by mimicking non-genomic steroid action, DHP activates both membrane-bound and nuclear progesterone receptors. This dual action enables comprehensive restoration of reproductive function in mutant females.

Additionally, the identification of critical gene markers such as adamts9 and adam8b provides valuable insights into the molecular mechanics of successful ovulation. These metalloproteinases, specifically expressed in granulosa cells surrounding the oocyte, facilitate follicle rupture – a process essential for egg release that fails in untreated mutants.

The practical implications of this research extend far beyond laboratory settings. Fish farmers can now maintain sterile populations with superior flesh quality while retaining the ability to restore fertility when needed. This controllable sterility strategy also addresses environmental concerns by preventing genetic contamination of wild populations through escaped farmed fish.

From a broader perspective, these findings establish zebrafish as an excellent model for understanding reproductive mechanisms across species. The conservation of key reproductive pathways between zebrafish and mammals suggests potential applications for human fertility treatments as well. Zebrafish research thus continues to prove invaluable for advancing both aquaculture innovation and reproductive medicine.

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