



# **Producing viable larvae of African catfish** *Clarias gariepinus* from in vitro matured and ovu died oocytes

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IVM medium:



- Translucent ooplasm



## IVM oocyte isolation: **IMMATURE OVARIAN FOLLICLES** Postvitellogenic, fully grown oocyte

**Opaque cytoplasm** Centrally postioned germinal vesicle (GV) Ø 1.0 - 1.2 mm



Hormonal stimulation of postvitellogenic ovarian follicles:

+ hCG (human chorionic gonadotropin) - 10-25 IU/ml

**DHP** (17α, 20β-dihydroxy-4-pregnen-3-one) - **10-1000 ng/ml** 

+ rhlGF-1 (recombinant human insulin-like growth factor 1) - 50-250 ng/ml



# - Germinal vesicle breakdown (GVBD)



# X 14.00

## **IN VITRO OVULATION**

- Hormonal stimulation of follicular layer
- rupture after IVM
- + **PGF2α** (prostaglandin F<sub>2</sub>α) **5-10 μg/ml**
- + PGE2 (prostaglandin E<sub>2</sub>) 5-10 μg/ml

# **IN VITRO MATURATION**



Progress of in vitro maturation during a 14-hour treatment with DHP (1  $\mu$ g/ml).



80

GVBD 99

**% 40** 

20

#### African catfish ovarian follicle after **DHP-induced in vitro maturation.**

The oocyte is surrounded by an intact follicular layer (not ovulated). An annular bulge formed on the animal pole surrounds the micropyle opening (arrowhead).

#### African catfish mature oocyte (egg) after PGF2a-induced in vitro ovulation.

The remnants of the follicular layer are attached to the animal pole (asterisk). After ovulation, an adhesive disc composed of many attaching filaments forms on the animal pole (arrow), while a thin mucous layer surrounds the oocyte surface (arrowhead).



Effects of hormones and culture media pH/supplementation on in vitro maturation. Stimulation with DHP (1  $\mu$ g/ml) is sufficient to induce high rates of GVBD, while co-treatment with hCG (25 IU/ml) or rhIGF-1 (250 ng/ml) does not influence the IVM outcome. Compared to results in media with pH of 7.5, significantly less follicles matured in a more alkaline environment (pH 8.5), regardless of supplement type and concentration.

The maturation process was marked by the change in the ooplasm optical density, as well as by the migration of GV (germinal vesicle; GV) towards the periphery and its subsequent dissolution (germinal vesicle breakdown; GVBD), which is a marker for meiosis resumption.

# **IN VITRO OVULATION**



0 h

Progress of follicular layer rupture and ovulation during a 2-hour treatment with PGF2 $\alpha$  (5  $\mu$ g/ml), following a DHP-induced in vitro maturation.



Visualization of follicular layer integrity during in vitro ovulation. Nuclei of follicular and thecal cells are stained with SYBR Green I fluorescent dye.









#### Effects of hormones and culture media pH/supplementation on in vitro ovulation.

Following stimulation with DHP (1µg/ml), only 4±3% of the mature oocytes ovulate in vitro. Incorporation of prostaglandins (PGs) as an additional incubation step following DHP-induced GVBD significantly promotes ovulation, most notably in groups with 5 µg/ml PGF2a. Compared to media with pH of 7.5, significantly less follicles ovulated in an alkaline environment (pH 8.5). Supplementation with FBS (5-10%) further promotes ovulation at pH 7.5.



Fertilization of and development of eggs and embryos of maturation and ovulation in vitro. In vitro matured and ovulated oocytes obtained in non-supplemented media (pH 7.5) maintained their developmental competence and were successfully fertilized. The hatching rate was 39%, after which the survival rate of larvae was 8% at 72 hours post fertilization (hpf).

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