Evaluation of dose of ovaprim for inducing ovarian maturation and ovulatory response in the catfish, Heteropneustes fossilis

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ABSTRACT
The present study was aimed to standardise the economically effective dose of ovaprim (OPm) which could induce oocyte development, growth, maturation and ovulation within optimum period in Heteropneustes fossilis. Mature females weighing 50±5 g of 15±2 cm in length were injected with variable doses of OPm (0.10, 0.15 or 0.20 ml/fish) during 2nd week of July and ovulation was checked at 8, 16, 20 and 24 h interval. The ovarian hydration was significantly high after 20 h at 0.10 and 0.15 ml dose levels and 8 h at 0.20 ml dose. In OPm injected fish, spawning was 65% at 24 h, 25% at 20 h and 75% at 16 h in 0.10, 0.15 and 0.20 ml dose levels, respectively. The ovulated eggs diameter was significantly more in the 0.20 ml OPm injected group compared to the other two. The number of ovulated eggs/fish was also significantly more in 0.20 ml OPm injected fish in comparison of 0.15 ml but the difference was insignificant with 0.10 ml OPm injected group. The histological observations revealed that ovaries of the control fish contained large number of yolky oocytes usually passing through various atretic stages. The follicular cells were not active and even the process of intravesicular yolk synthesis in vitellogenic oocytes was not marked. In all OPm injected groups, dose and duration dependent development, growth and maturation of oocytes were superior at higher dose which included proliferation of primordial cells forming oogonial cyst, differentiation of follicular layer into active thecal and granulosa cells, induction of intra and intervesicular yolk synthesis and incorporation, advancement of maturational events in fully grown oocytes i.e. germinal vesicle movement and germinal vesicle breakdown, separation of follicular wall and ovulation. Above observations suggested that 0.20 ml/fish dose of OPm was most effective in inducing oocyte growth and maturation, however, 0.10 ml/fish of OPm on the basis of spawning response was though slightly delayed in comparison of 0.20 ml, which might be considered as economically appropriate dose for inducing ovulation and spawning in this species.

Introduction
The catfish H. fossilis is an economically important food fish of India and enjoys higher price and demand in the market owing to its nutritive, invigorating and therapeutic qualities (Day, 1989). Its flesh is rich in protein and iron but poor in fat content, hence, physicians recommend it as diet during convalescence. Due to its air-breathing habit, it thrives...
well even in oxygen deficient derelict and vegetation infested water bodies, and is ideal for wastewater aquaculture. It permits high stocking density (4000-6000 fry/ha) in well managed ponds, grows to marketable size in 6-8 months. Its availability has been dwindling during the last two decades, being totally dependent upon capture fisheries and regular recurrence of epizootic ulcerative syndrome (Das, 1997). Despite many high positive attributes, its culture is not popular mainly because of non-availability of sufficient stocking material. In view of the limited scope of its seed collection from natural resources, seed production by induced breeding is the only other option for ensuring availability of seed.

For the first time, induced breeding of H. fossilis was accomplished with pituitary extract by Ramaswamy and Sundararaj, 1956. The first concerted effort for developing a technique for its breeding was taken up from 1971 to 1985 under “All India Co-ordinated Research Project on Air Breathing Fish Culture” at various centers (Workshop Report, 1987). Meanwhile, efforts were made by several workers for inducing ovulation / spermiation / breeding of this fish using various inducing agents i.e. mammalian gonadotropin luteinizing hormone (LH) by Anand and Sundararaj (1974), partially purified salmon gonadotropin (SG - G100) by Sundararaj et al. (1971, 72), homologous and heterologous pituitary extract by Singh et al. (1982), salmon gonadotropin releasing hormone analogue (sGn RH-A) and domperidone alone or in combination by Alok et al. (1993), mammalian gonadotropin releasing hormone analogue (mGn RH-A) and pimozide alone and in combination by Tharakan and Joy (1996).

After its advent, ovaprim (OPm), a synthetic form of sGn RH-A (D-Arg6-Pro9-N ethylamide) in combination with domperidone, a dopamine antagonist, has taken the place of the pituitary extract. However, the effectiveness of ovaprim as a spawning inducing agent, has not been successfully evaluated for catfishes. Therefore, an attempt has been made in the present investigation to standardize the dose of ovaprim which could suitably induce the developmental and maturational events in ovary.

**Materials and methods**

Sexually mature specimens of H. fossilis, weighing 50±5 g and measuring 15±2 cm in length, were collected during the last week of June from local fish pond in Pantnagar and acclimated to the laboratory conditions for a fortnight period. No feeding was done during the 1st week of acclimatization; 2nd week onwards the fish were fed with minced goat liver ad libitum daily. Water was changed daily after feeding. Male and female specimens were sorted out on the basis of their morphological characteristics (Singh, 1998).

Females were divided into four groups of 30 each, maintained separately in large plastic pools containing 70 litre chlorine free deep well water. The first group was kept as control and injected with 0.20 ml/fish of 0.6% (NaCl) normal saline. Remaining three groups (II, III & IV) were given ovaprim injection of 0.10, 0.15, and 0.20 ml/fish respectively. In all three groups ovulation was checked at 8, 16, 20 and 24 h intervals by hand stripping. The abdomen was squeezed gently from the anterior towards the ovipore and the fish that yielded a copious stream of transparent green brown eggs were rated as ovulated. The maturation status of ovulated eggs was assessed by determining the position of germinal vesicle after cleaning the ooplasm for 10 minutes with a mixture of methanol, ethanol and acetic acid solution (1:1:1-V/V) adopting the methods described by Crim and Glebe (1990). The measurement of ova diameter was done with the help of ocular-stage micrometer.
For the purpose of histophysiologi-
cal observation, five non-ovulated speci-
mens from each group at 8, 16, 20 and 24
h intervals were sacrificed by decapita-
tion and ovaries were dissected out and
fixed in aqueous Bouin’s fluid. Paraffin
sections were cut at 5 µm thickness and
stained with Ehrlich’s haematoxyline
using eosin as a counter stain. For the
estimation of water content, parts of ova-
ries were collected from specimens sacri-
ficed at 8, 16, 20 and 24 h intervals. Sam-
ple were weighed by electronic balance
to record initial weight. These samples
were dried in oven at 55±5°C for at least
2 days and again weighed to have final
dry weight for calculation of percent wa-
ter content. The numerical data were sub-
jected to Student’s t-test for evalua-
tion of level of significance (P-
value).

Results

The results of ovaprim injected H.
fossilis on dose and duration dependent
spawning responses, ovarian hydration,
frequency of ovulated eggs and ova di-
ameter are presented in Table 1.

(I) Control

As the experiment was conducted
during the 2nd week of July, i.e. spawn-
ing phase of its reproductive cycle, ova-
ories of the control fish were full of large
number of yolky oocytes with centrally
placed germinal vesicle, a few vitellogenic
and immature oocytes. Some of the yolky
oocytes in various stages of atresia and
remnence of degenerated oocytes were
also seen (Fig. 1). Although, the yolky
oocytes contained sufficient quantity of
yolk material and were encapsulated by
the follicular layer, follicular cells were
not active as evidenced by their fibrous
nature. There was no initiation of germi-
nal vesicle movement (GVM) or germi-
nal vesicle breakdown (GVBD) resulting
in total failure of ovulation (Fig. 2).

(II) OPm injected group
At 8 h interval

In the experimental group of fish
injected with any three doses of OPm, no
change was noticed in the growth of
vitellogenic oocyte and also in matura-
tion of yolky oocytes after 8 h in response
to lower dose of 0.10 ml/fish. However,
Table 1. Dose and duration dependent spawning responses, ovarian hydration, frequency of ovulated eggs and ovadiameter in *Heteropneustes fossilis* injected with different doses of ovarpim (OPm). Values are mean ±SD of five specimens in each group. Percent spawning values are expressed in terms of number of fish ovulated in each group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>OPm Injected Group (ml/fish)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration (Hours)</td>
<td>0.10</td>
</tr>
<tr>
<td>% Spawning</td>
<td>08</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>% Water Content in Ovary</td>
<td>08</td>
<td>52.40±3.174 (NS)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>52.68±3.729 (NS)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>51.74±3.120 (P&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>53.43±2.772 (P&lt;0.05)</td>
</tr>
<tr>
<td>Number of Ovulated Eggs/fish</td>
<td>Nil</td>
<td>3028±290</td>
</tr>
<tr>
<td>Ovulated Egg Diameter (mm)</td>
<td>Nil</td>
<td>1.771±0.1178</td>
</tr>
</tbody>
</table>

P = Level of significance of OPm injected group compared with respective control group.
P1 = Level of significance of 0.10 ml OPm injected group compared with 0.15 ml OPm injected group.
P2 = Level of significance of 0.20 ml OPm injected group compared with 0.15 ml OPm injected group.
P3 = Level of significance of 0.20 ml OPm injected group compared with 0.10 ml OPm injected group.
NS = Statistically ‘Not significant’.
Ovaprim induced maturational and ovulatory response in H. fossilis

towards the animal pole side, GVM and GVBD (Figs. 5 & 6). The cyst of new oogonials and increase in number of immature and some vitellogenic oocytes could also be witnessed (Fig. 5). Further, the process of GVBD could be distinguished by the formation of conspicuous space arraying from germinal vesicle towards the animal pole side possibly due to coalescence of yolk globules, break down of germinal membrane and dispersal of germinal material (Fig. 7). In such oocytes, the appearance of cortical alveoli in the periphery of ooplasm close to the distinct vitelline membrane was also indicative of induction of the terminal events of maturation leading towards ovulatory phase (Fig. 8).

Although, in any of the above OPm injected groups, spawning was not recorded at 8 h interval, percent water content of the ovary in 0.20 ml OPm group.

Fig.2. Inset part of figure 1 further magnified showing a yolky oocyte with centrally placed germinal vesicle (GV) and ooplasm full of yolk material. H&E X225.

Fig.3. Ovary of fish injected with 0.10 ml/fish OPm after 8 h interval with full of yolky (*) and group of immature (>) oocytes. H&E X45.

Fig.4. Ovary of fish injected with 0.15 ml/fish OPm after 8 h interval containing large number of vitellogenic (Vg) and immature (>) oocytes, oogonials (>) and a few yolky oocytes (*). Huge debris-mass (X) of atretic oocytes could also be seen. H&E X45.
was significantly more (P<0.05) when compared to control.

**At 16 h interval**

After 16 h interval, the fish group injected with the lowest dose (0.10 ml/fish) showed large number of fully grown yolky oocytes at the stage of completion of GVM and initiation of GVBD as char-

Fig.5. Ovary of fish injected with 0.20 ml/fish OPm after 8 h interval exhibiting the presence of large number of fully grown yolky oocytes (*) under maturational processes i.e. coalescence as well as accumulation of yolk bodies towards vegetal pole (➞) and GV on the animal pole (➞). Group of oogonial, immature and Vitellogenic (Vg) oocytes could also be seen. H&E X45.

Fig.6. Ovary of above mentioned experimental group containing large number of fully-grown oocytes with completion of GVM (➢) as well as GVBD (➞) oocytes. H&E X45.

Fig.7. Part of the mature oocyte in inset (a) of figure 6 enlarged to show the formation of conspicuous space (➞) arraying from breaking germinal vesicle (➞). H&E X450.

acterized by the development of conspicuous space surrounding the GV, possibly due to coalescence of yolk globules and irregular wavy shape of the germinal membrane (Figs. 9 & 10). The histophysiological organization of ovary in 0.15 ml/fish injected group revealed further advancement of growth possibly in those vitellogenic oocytes which were earlier under the process of growth phase at 8 h interval (Fig. 11). In such oocytes the initiation of GVM but not GVBD as evi-
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denced by the eccentrically positioned GV and development of germinal crest in its
close proximity was observed (Fig. 12). The number of immature oocytes was
also same as in 8 h hour group suggesting simultaneous activation of oogonial
proliferation (Fig. 11).

On the other hand in 0.20 ml/fish injected group, ovaries from unspawned
specimens contained more number of fully grown yolky oocytes in which final
maturation was completed (Fig. 13).

Moreover, the presence of significant number of immature and vitellogenic
oocytes were also suggestive of sustained oogonial proliferation and growth activities in ovaries. Due to completion of final maturation events in number of oocytes
and significant augmentation in ovarian water content (P<0.05), 75% spawning
was recorded in this group at this interval. However, in 0.15 ml/fish dose significant increase in ovarian water content (P<0.05) was recorded at 20 and 24 h intervals resulting in feeble spawning (25%) at 20 h. Although, in specimens of 0.10 ml/fish dose groups ovarian water content was significantly augmented (P<0.05) at and after 20 h intervals, significant spawning was recorded only at 24 h.

**At 24 h interval**

At this interval, the ovaries of 0.10 ml injected unspawned fish were containing large number of fully grown yolky oocytes at the threshold stage of maturation (Figs. 14 & 15). Similarly the ovaries of 0.15 ml injected fish were full of large number of fully grown yolky oocytes passing through various maturation stages as characterized by eccentric position of GV (Fig. 16). These observations indicating delay in maturation processes could be the reason for the late spawning response at 20 h interval in 0.15 ml dose group and at 24 h interval in 0.10 ml dose groups. On the other hand, the ovaries of fish from 0.20 ml injected group were containing oocytes in all stages of maturation with completion of GVBD (Fig. 17). In the ovaries of some fish, oocytes at different stages of GVM and GVBD were also seen (Fig. 18). In the oocytes of growth phase, follicular layer was well developed and differentiated into thecal and granulosa cells exhibit-
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...ing incorporation of intervesicular yolk material (Fig. 19) attributed to the activation of vitellogenesis too.

The number of ovulated eggs/fish was significantly higher in 0.20 ml group (P<0.01) when compared with 0.15 ml group but with insignificant difference in comparison of 0.10 ml group. Moreover, number of ovulated eggs/fish was also more for lowest dose group compared with medium dose group but with insignificant difference. Correspondingly the ova-diameter increased significantly in higher dose (0.20 ml/fish) group (P<0.01) compared with 0.10 ml and 0.15 ml groups. However, the difference was insignificant between medium and low dose groups. These correlative observations of gamete quality are suggestive of superiority of higher dose level.

**Discussion**

The results revealed that in different treatments, ovulation was not noticed in any of the groups before 16 h since treatment. However, the histophysiologica observations indicated that 0.10-ml treatment induced growth and maturatio processes in vitellogenic and yolky oocytes, whereas, 0.15 ml dose was able to induce oogonial proliferation, growth of vitellogenic oocytes and also maturation processes in vitellogenic and yolky oocytes.

As far as ovulatory responses are
concerned it was 75% at 16 h in 0.20 ml/dose group, 65% in 0.10 ml at 24 h, whereas, it was only 25% at 20 h gap in 0.15 ml treated group. However, after 24 h the histophysiological observations of unspawned specimens indicated that in all experimental groups, ovaries had large number of yolky oocytes at various maturation stages with significant increase in ovarian water content.

The number of ovulated eggs/fish was significantly more at higher dose when compared with medium dose but not with low level. It signified the effectiveness of low dose under longer and high dose under short responsiveness period. Pandey and Singh (1977) have reported corroborative observations on effectiveness of low dose of OPm in inducing spawning of Indian major carps. On the other hand, ova-diameter was sig-
The present study suggested that 0.20 ml/fish dose of ovaPrim was capable of inducing growth and maturation events in all type of oocytes even after lapse of short time interval and continued for longer duration which helped in maintaining the fecundity potency of ovary for prolonged duration. As a result, there was pronounced spawning at 16 h interval. The 0.10 ml/fish dose induced maturation process only in growing yolky oocytes at slow pace resulting in significant ovulation at 24 h interval. However, the medium dose of 0.15 ml/fish was less effective in inducing terminal ovulatory events resulting in the formation of atretic oocytes though it had induced successful maturation events in yolky oocytes during early period of its administration i.e. after 8 h. Hence it can be inferred that 0.10 ml/fish dose is economically appropriate and 0.20 ml/fish the most effective dose for the inducement of ovarian final maturation. Earliest spawning was recorded in 0.20 ml dose after 16 h and 20 h or more in other doses. In H. fossilis, mammalian gonadotropin releasing hormone analogue (mGnRH-A) alone or in combination with pimozide have been reported to cause pre-ovulatory surge in plasma gonadotropin and a high rate of ovulation. Low dose of mGnRH-A when given alone was not effective in the early (July) but effective in the late (August) period of spawning season. The upsurge in plasma gonadotropin level in above treatments have been reported to vary according to period of reproductive cycle (Tharakan and Joy, 1996). These observations emphasize that gonadal status of the recipient fish or the period of its breeding season plays vital role in responding to the inducing agents administered exogenously, and their dose have to be decided accordingly.

The latency period between injection and spawning is comparatively high in H. fossilis. By using a combination of mGnRH-A and pimozide ovulation was recorded after 12 h in H. fossilis during August (late spawning period) but not in July (early spawning period) (Tharakan and Joy, 1996). Similar spawning responses in the same species have been observed 24 h after the injection of heterologous and homologous pituitary extracts (Singh et al., 1982). These reports are in conformity with the observations made in the present study that H. fossilis requires comparatively high doses of inducing agents which varies according to reproductive status and longer latency period between injection and ovulation.

References


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