

## Endocrine sex reversal in fishes: Masculinization evokes greater stress and mortality

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In fishes the hormonal induction of masculinization is a more stressful process (as evidenced by higher mortality) than that of feminization. This is further evidenced from observations reported for all-male and all-female triploids. The presence of sex-determining chromosome Y in pair appears to render the induction of endocrine sex reversal a more stressful process. The reproductive performance of neofemales especially those subjected to sex reversal by synthetic estrogens in *Betta splendens* is not equal to that of genetic females.

In fishes sex is genetically determined; however, the administration of one or the other sex steroid is known to induce sex reversal. The process of sex reversion comprises the entire sequence of differentiation, gametogenesis, ovulation/spermiation, courtship behaviour, secondary sexual characteristics and sex pheromone production<sup>1</sup>. Ever since Yamamoto<sup>2</sup> artificially induced sex reversal in genetic males of medaka, *Oryzias latipes* numerous papers have been published and have been reviewed from time to time<sup>1,3,4</sup>. The administration of a critical dose of a sex steroid, say  $\beta$ -estradiol during the labile period reverses the phenotypic expression of genetic male into female but genetic female remains a female<sup>5</sup>. Since the performance of this sex-reversed female is almost comparable to genetic female, the genotype of this neofemale can be identified only by progeny testing. Although many scientists have successfully reserved the sex by administering one or the other steroid, very few have adopted progeny testing to confirm the genotype of the sex-reversed females or males<sup>6</sup>. This may be because progeny testing requires long-term experiments. In our laboratory we undertook progeny testing and compared the survival of the genetic and sex reversed males and females with that reported by previous authors<sup>5,7</sup>. This study revealed that the survival of the neofemales (genetic males) was lower than that of the genetic females. The survival of neomales (genetic females) was even lower, and that of YY neosuperfemales was the lowest. This paper reports on the survival and performance of hormonally sex reversed male and female fishes. As the progeny testing involved only the sexually mature individuals, it was possible to quantify the proportion of these neofemales and neomales, which survived until breeding. Since these individuals were subjected to more or less

the same experimental conditions, a greater mortality of, say, neofemale any time from the treatment period until breeding, would reflect the stress suffered by the genetic male, which had been reversed to perform the function of a female.

Table 1 summarizes the available information on the sex ratio in fishes that were subjected to endocrine sex reversal and subsequently to progeny testing. It includes data reported for feminization in oviparous species *Betta splendens*<sup>5</sup> and *Oreochromis mossambicus*<sup>7</sup> as well as viviparous guppy *Poecilia reticulata*<sup>8</sup>. It also includes information on masculinization in oviparous species of *Salmon gairdneri*<sup>9</sup>, *B. splendens*<sup>10</sup> and viviparous *P. reticulata*<sup>11</sup>. Information has also been reported for sex ratio in masculinized *Tilapia aurea*<sup>12</sup>, in which females are heterogametic<sup>13</sup>. We report data on the survival of (YY) supermales and (YY) neosuperfemales.

Observations on feminization show that the percentage composition of genotype within each of these endocrine treated, breeding populations was tilted in favour of genetic females. Survival of the neofemales is lower (mean 40%) than that (mean 60%) of the genetic females in all four tested species, in which sex reversal was induced with estrogens. Likewise the survival of neomales, when treated with androgens was lower (mean 19%) than that (mean 81%) of the genetic males. This is particularly true of the viviparous *P. reticulata*, in which sex reversal could successfully be induced, only when the selected androgen was administered at a very high dose. Whereas 40% of the neofemales survived to breed, only 24% of the neomales survived to breed (Table 1). Therefore the endocrine induction of masculinization seems to be more stressful than that of feminization. It is not clear whether there is built-in mechanism, which favours feminization<sup>15</sup>. However more information is urgently needed before a generalization can be made.

Incidentally, it is interesting to compare the data reported for the production of all-female or all-male triploids. In an attempt to produce all-female triploids in *O. mossambicus*, we<sup>16</sup> crossed the hormonally sex reversed neomales (XX) with normal females (XX) and produced all-female triploids (XXX) by shocking the fertilized eggs to retain the second polar body. The survival of these females was  $59 \pm 6\%$ . In another case, Kavumpurath and Pandian<sup>17</sup>, produced all-male fighterfish by crossing the hormonally sex reversed neofemales (XY) with normal males (XY), and retaining the second polar body; therefore they produced triploid males carrying XXY and XYY genotypes; as these triploids were sterile, it was not possible to trace their genotype by progeny testing. Remarkably the survival of these all-male triploids (XXY and XYY) was  $45 \pm 7\%$ . Therefore these processes, which involved both endocrine treatment and ploidy induction, led to the production of triploids,

whose survival was lower for males than females. These observations appear to confirm our conclusion that induction of masculinization is a more stressful process in these species, in which males are heterogametic.

Remarkably, the survival of neomales of *T. aureus*,

**Table 1.** Percentage sex composition of fishes which were subjected to endocrine sex reversal. Genotype of the sex reversed fish was identified by progeny testing

Species and ref.	Steroid	Effective minimum dose (mg/kg food)	Survival (%)	
			Neo-females (XY)	Fe-males (XX)
<b>I. Heterogametic male</b>				
<i>Feminization</i>				
<i>B. splendens</i> <sup>5</sup>	β-Estradiol	125	44	56
	17 α-Ethynyl-estradiol	50	30	70
	Diethylstilbestrol	20	37	63
<i>O. mossambicus</i> <sup>7</sup>	Diethylstilbestrol	100	55	45
<i>P. reticulata</i> <sup>8</sup>	β-Estradiol	400	31	69
	17 α-Ethynyl-estradiol	200	39	61
	Diethylstilbestrol	300	44	56
Mean survival (%)			40	60
<i>Masculinization</i>				
<i>B. splendens</i> <sup>10</sup>	19-Norethisterone acetate	8	80	20
	17 α-ethynyl-testosterone	15	70	30
	Androstenedione	90	62	38
	11 α-Ketotestosterone	60	80	-
<i>Salmo gairdneri</i> <sup>9</sup>	17-Methyl-testosterone	1	85	15
<i>P. reticulata</i> <sup>11</sup>	19-Norethisterone acetate	300	83	17
	17 α-Ethynyl-testosterone	500	90	10
	Androstenedione	200	58	42
Mean survival (%)			76	24
<b>II. Heterogametic female</b>				
<i>Masculinization</i>				
<i>Tilapia aurea</i> <sup>12</sup>	17 α-Ethyl-testosterone	30	63	27
	17α-methyl-testosterone	30	54	46
	Mean survival (%)		59	37

in which the females (WZ) are heterogametic, is lower than that of genetic males, when they were masculinized. It is also not clear whether masculinization is a more stressful process in species in which females are heterogametic. Lincoln<sup>18</sup> reported the survival values for the triploid plaice *Pleuronectes platessa*, in which the female is heterogametic (WZ). Survival values are 95% for males (ZZZ) and 5% for females, whose genotypes could have been WWZ or WZZ. On the whole, observations indicate that the presence of sex-determining chromosome (Y or W) in pair appears to render the induction of endocrine sex reversal a more stressful process.

As the presence of Y chromosome in pairs appears to inflict a greater mortality in the sex-reversed individuals<sup>19</sup>, we analysed the available data on the percentage mortality on supermale (YY) and hormonally sex-reversed neosuperfemales (YY). For the production of supermales and neosuperfemales, sex-reversed neofemales (XY) are first produced by estrogen treatment at F<sub>1</sub>; in the oviparous species, this is followed by the induction of gynogenesis, as in *O. mossambicus*<sup>7</sup>, or selective breeding, as in other cases (Table 2). If the first procedure is followed, only homogametic males (50% YY) and females (50% XX) are produced. However, if the second procedure is adopted, 25% homogametic females (XX) and 50% heterogametic males (XY) and 25% homogametic males (YY) are produced<sup>7</sup>. Unfortunately, the survival data for these females (XX) are not immediately available (Table 2; item ii a to c). However, the values recorded for the survival of homogametic males (YY) ranged between 4 and 17%, which are far lower than those obtained for the normal heterogametic males (XY).

The production of homogametic neosuperfemales (YY ♀) and heterogametic neofemales (XY ♀) among F<sub>2</sub> progenies involves endocrine treatment for feminization

**Table 2.** Percentage sex composition of fishes, which were subjected to endocrine sex reversal (F<sub>1</sub> generation) and gynogenesis\* or selective breeding\*\* (F<sub>2</sub> generation). Genotype of sex reversed fish was identified by progeny testing

Species and ref.	Genotype		
	Genotype of F <sub>2</sub> male (%)		
	(XY)	(YY)	
i) <i>Oreochromis mossambicus</i> <sup>5</sup>	*	30	
ii) <i>Carassius auratus</i> <sup>20</sup>	83**	17	
ii) <i>Oryzias latipes</i> <sup>3</sup>	96**	4	
iii) <i>Poecilia reticulata</i> <sup>11</sup>	89**	11	
	Genotype of F <sub>2</sub> females (%)		
	XX	XY	YY
iii) <i>O. latipes</i> <sup>3</sup>	50	29	21
ii) <i>P. reticulata</i> <sup>8</sup>	65	26	6

\*In this method, no heterogametic males are produced

\*\*In this method 50% heterogametic males are produced (see also Figures 1 and 2)

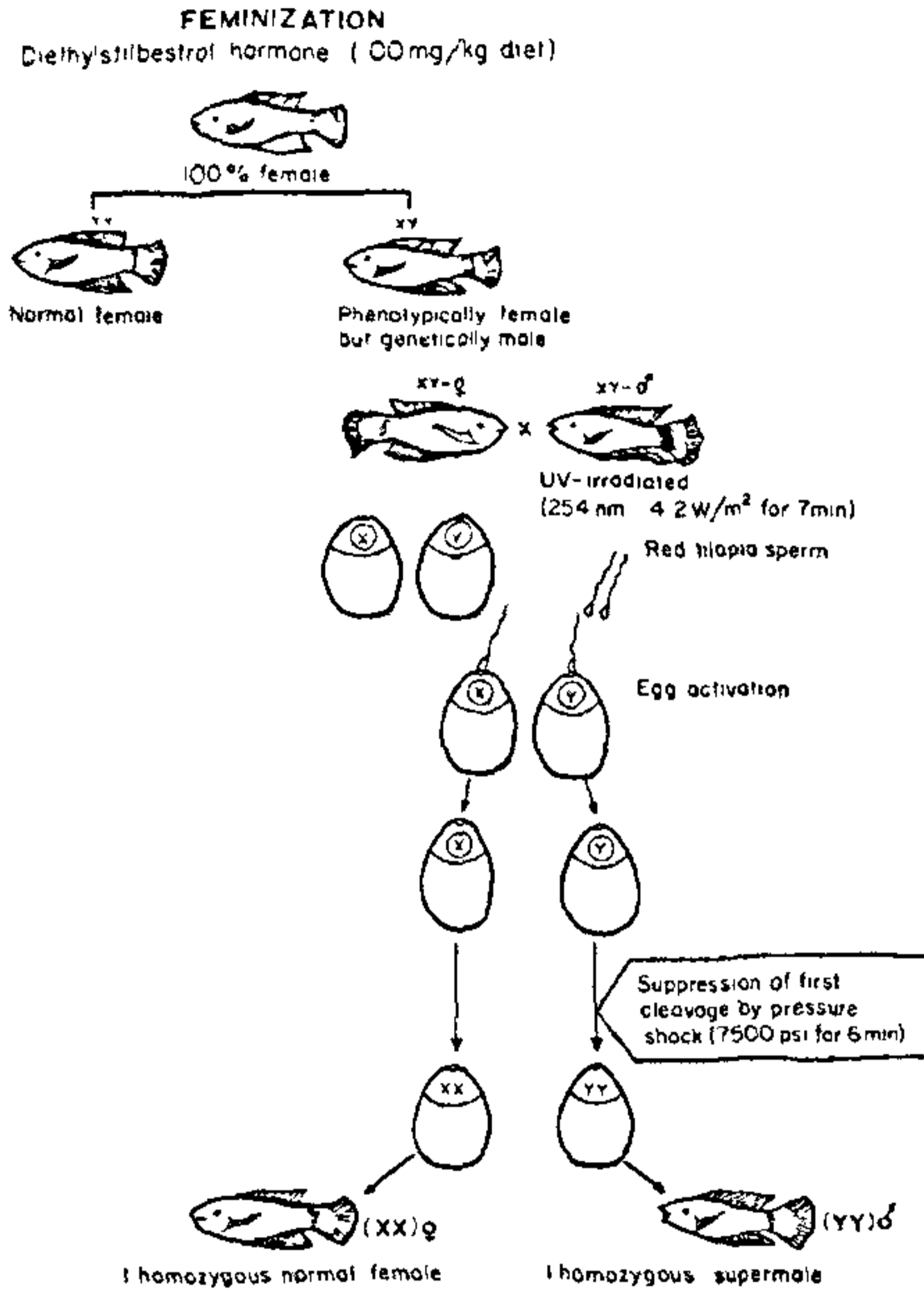


Figure 1.

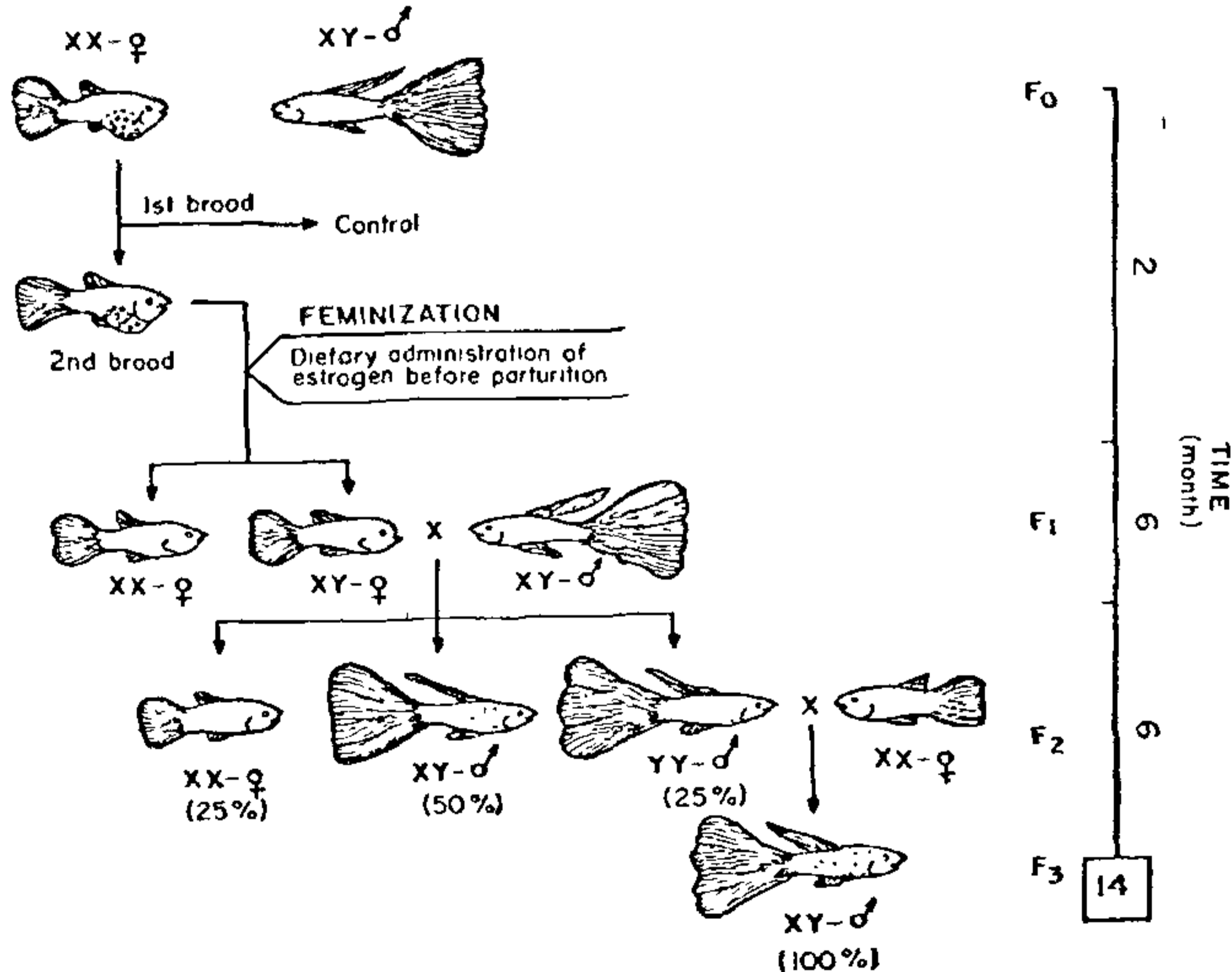


Figure 2.

Table 3. Functional equality of normal and sex-reversed female *Betta splendens* (from Kavumpurath and Pandian, 1993a, modified)

Females	Age at puberty (days)	Fecundity (eggs/spawning)
Control XX ♀♀	178 ± 14	516 ± 58
Treated with β-estradiol		
XX ♀♀	198 ± 19	485 ± 77
XY ♀♀	194 ± 10	487 ± 18
Treated with 17α-ethynylestradiol		
XX ♀♀	210 ± 15	525 ± 30
XY ♀♀	232 ± 24	418 ± 15
Treated with diethylstilbestrol		
XX ♀♀	219 ± 31	405 ± 62
XY ♀♀	241 ± 29	425 ± 63

twice, the first one involving the fertilized viviparous females, or the progenies of normal (XX ♀ and XY ♂) parents, in the case of oviparous species, and the second one involving the fertilized (by XY ♂) XY neofemale, as in *P. reticulata* or the progenies of the cross between XY ♀ and XY ♂ parents, as in *O. latipes*. Such long term experiments involving progeny testing have so far been undertaken only in two species, but fortunately involving an oviparous *O. latipes* and a viviparous *P.*

*reticulata* (Table 2). Clearly the survival values reported for these heterogametic neofemales were lower than those obtained for homogametic females. The values obtained for the homogametic neosupermales were even lower than those obtained for neofemales. Remarkably the survival values reported for sex reversed YY females of *P. reticulata* were the lowest, perhaps due to the fact that these viviparous females were treated with high estrogen dose.

It appears that the hormonally sex reversed females not only suffer high mortality but are also not functionally equal to genetic females. As an example, the observations made by Kavumpurath and Pandian<sup>5</sup> for *B. splendens* is presented here. In this species sex reversal was ensured using the natural and synthetic steroids ( $\beta$ -estradiol and 17  $\alpha$ -ethynylestradiol). In all these neofemales, the egg size ( $\approx 1000\mu\text{m}$ ) and the hatching success (80.5%) were comparable to those observed for the control. However, the age, at which the neofemales attained sexual maturity, was significantly postponed, especially in XY neofemales; they also produced significantly less number of eggs per spawning than the genetic females, or XX or XY females that were hormonally treated with natural steroid  $\beta$ -estradiol (Table 3). Therefore, it appears that the neofemales, that were treated with synthetic steroids, were not functionally equal to normal females, as the neofemales suffer from delayed maturity and reduced fecundity. This paper reports for the first time the lower percentage of survival and poorer reproductive performance of hormonally sex reversed neofemale fishes.

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