Our results corroborate the existence of biased eggs in this species. Moreover, they lead us to believe that caste determination is tied to ecdysteroid titre. There appears to be a level below which eggs are sexually-biased, as is the case just after hibernation and above which they are worker-biased. Variations in ecdysteroid titre have also been recorded in *Macrotermes* termites eggs[9] but their destiny is as yet unknown. Also, after caste determination has taken place, the sexually- and worker-biased larvae of honeybees[12] exhibit significantly different ecdysteroid levels.
The roles of JH and ecdysteroids in caste determination can be compared. JH is already known to spur brood sexualization in *Apis*[13] and, in the present case, in *Pheidole*[3]. In *Apis*[14], a great JH increase coincides with caste determination of queen larvae. In *Polistes*[10], JH stimulates oocyte development whereas ecdysterone seems to inhibit it.
A hypothesis based on relative variations of JH and ecdysteroids has already been proposed for termites[15,16]. So it would not be unprecedented if, during caste determination in social insects, a high JH titre accompanies queen biasing while a high ecdysteroid titre goes with worker biasing.

1 L. Passera, Insectes soc. *21*, 67 (1974).
2 L. Passera, Insectes soc. *26* (1979).
3 L. Passera and J.P. Suzzoni, C. r. Acad. Sci. Paris *287*, D, 1231 (1978).
4 M. De Reggi, M. Hirn and M. Delaage, Biochem. biophys. Res. Commun. *66*, 1307 (1975).
5 H. Hagedorn, J. O'Connor, M. Fuchs, B. Sage, D. Schlaeger and M. Bohm, Proc. natl Acad. Sci. USA *72*, 3255 (1975).
6 M. Lagueux, M. Hirn and J. Hoffmann, J. Insect Physiol. *23*, 109 (1977).
7 T. Hsiao and C. Hsiao, J. Insect Physiol. *25*, 45 (1979).
8 D. Bullière, F. Bullière and M. De Reggi, Wilhelm Roux's Arch. *186*, 103 (1979).
9 J.P. Delbecque, B. Lanzrein, C. Bordereau, H. Imboden, M. Hirn, J.D. O'Connor, C. Noirot and M. Lüscher, Gen. comp. Endocr. *36*, 40 (1978).
10 A. Strambi, C. Strambi and M. De Reggi, Proc. 8th Congr. IUSSI, p. 19. Wageningen, 1977.
11 A.R. Gande and E. Morgan, J. Insect Physiol. *25*, 289 (1979).
12 H. Hagenguth and H. Rembold, Mitt. dt. Ges. allg. angew. Ent. *1*, 296 (1978).
13 P. Wirtz, Meded. LandbHoogesch. Wageningen *73-5*, 1 (1973).
14 Y. Lensky, J.C. Baehr and P. Porcheron, C. r. Acad. Sci. Paris *287*, D, 821 (1978).
15 M. Lüscher, in: *Phase and Caste Determination in Insects*, p. 91. Ed. M. Lüscher. Pergamon Press, New York, 1976.
16 C. Noirot, Proc. 8th Congr. IUSSI, p. 12. Wageningen, 1977.

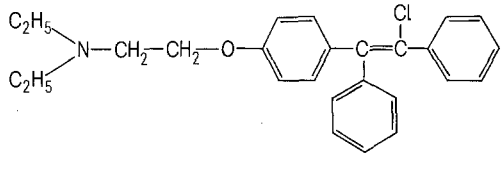
Artificial spawning effected in the fresh water teleost, *Cyprinus carpio* by clomiphene citrate

S. Kumar and K. Chandrasekhar[1]

Surgical Research Laboratory, Institute of Medical Sciences, Banaras Hindu University, Varanasi-5 (India), 15 May 1979

Summary. Triweekly i.m. injections of clomiphene citrate (group 1, 25 µg/0.5 ml and group II, 50 µg/1.0 ml) were administered for a period of 3 months during the preparatory period to female fresh water teleosts exhibiting ovarian recrudescence, while a control group received 0.5 ml of physiological saline throughout the period of experimentation i.e., from February through April. 50 µg clomiphene citrate treatment brought about a steady increase in ovarian size, and oocytes began to enlarge and mature and finally ovulation took place in April. This is 4 months ahead of their normal occurrence.

Chemistry of clomiphene citrate. Clomiphene citrate (I-p-diethylaminoethoxy) phenyl-I, diphenyl-2 chloroethylene citrate) which is marketed as Clomid® is an established drug for ovulation in human beings. Its structural formula is



For the past decade or so, much work has been done to establish the role of clomiphene citrate in the induction of ovulation in human beings and laboratory mammals. Its mode of action has been explained by a number of workers; to cite a few instances[2-4] in rats and[5-11] in human females. The findings of these authors and many others have proved beyond doubt that this drug is a powerful inducer of mammalian ovulation. But surprizingly only an isolated reference[12] exists as far as its action on fishes is concerned. This coupled with the fact that artificial induction of ovulation using chemical inducers is an important aspect of fisheries development, prompted the authors to undertake the present investigation.

About 200 fishes, more or less of the same weight and length, were collected from local ponds in Varanasi (India) and were acclimatized to laboratory conditions. During this period they were fed on liver slices. The photoperiod was maintained 10 light h/10 dark h throughout the tenure of the experimentation. The fishes were then divided into 3 groups. Group I received fish saline 0.5 ml and formed the control group. Group II and III received 25 µg/0.5 ml and 50 µg/0.5 ml of clomiphene citrate respectively and formed the experimental groups. Clomid was administered i.m. thrice a week on alternate days for a period of 3 months from February through April (the normal preparatory phase of the fish[13]). Fortnightly sacrifises of 5 fishes from each group were made to obtain material for histochemical studies. However, prior to sacrificing, the fishes were examined to see whether they spawned by stroking their abdomen backwards. It was observed that the control fishes did not spawn on day 90 while the 50 µg-treated fishes started spawning from day 60 onwards and the 25 µg-treated fishes started spawning from day 75 onwards.
Histological examination of the ovaries revealed that in the control fishes the ovaries contained mostly stage I and II oocytes while in the experimental groups the ovaries had already become filled with ripe ova. The 50 µg dose of the drug proved more effective in that it brought about earlier spawning. The existing data[12] brings out its action on

Changes in ovary after clomiphene citrate administration (Mean ± SD)

Time of sacrifice (month)	Mean wt of ovary/100 g b.wt (mg)			Percentage of nature oocytes			Diameter of nature oocytes			Percentage of atretic oocytes		
	Control	25 µg clomid	50 µg clomid	Control	25 µg clomid	50 µg clomid	Control	25 µg clomid	50 µg clomid	Control	25 µg clomid	50 µg clomid
15 days (February)	625±205	575 ±175	345 ±141	8.5	17*	30*	225±35	325 ±56	355 ±29	4	5*	6.5**
30 days (February)	650±225	1675 ±345	1595 ±150	15	29*	45*	226±42	495 ±52	475 ±35	5	7*	5**
45 days (March)	1155±175	2625*±305	3695**±225	17	39*	60*	322±58	575*±45	625**±72	3	7.5*	7.2**
60 days (March)	1485±275	2048*±125	5725**±250	16	55*	75*	370±52	625*±41	905**±86	6	6.0*	9.5**
75 days (April)	2095±315	3275*±276	8925**±275	13	85*	105**	395±39	972*±32	1025**±75	4	5*	12.5**
90 days (April)	2548±325	4525*±700	9845**±310	20	98*	125**	325±21	1195*±85	1315**±55	5	7.5*	14.5**

* $p<0.05$ Significant increase c.f. corresponding controls. ** $p<0.05$ Significant increase c.f. corresponding controls and 25 µg administrations.

'gravid' fishes, while in this study it is shown that Clomid can be effectively used during the regressed or 'off-season' and the fishes can be made to spawn about 4 months in advance of their normal breeding period (table).

1 The authors wish to express their gratitude to Richardson Merrel Co. for supplying the sample of clomiphene citrate, and Dr K.N. Udupa, Institute of Medical Sciences, Banaras Hindu University, Varanasi for facilities.

2 S. Roy, V.B. Mahesh and R.B. Greenblatt, Acta endocr. *47*, 669 (1964).
3 A.P. Labhsetwar, J. Endocr. *47*, 481 (1970).
4 S. Roy, R.B. Greenblatt and V.B. Mahesh, Fert. Steril. *15*, 310 (1964).
5 S. Roy, R.B. Greenblatt, V.B. Mahesh and E.C. Tungok, Fert. Steril. *14*, 575 (1963).
6 N. Kase, A. Mrouch and L.E. Olson, Am. J. Obstet. Gynec. *98*, 1037 (1967).
7 R.W. Kristner, Am. J. Obs. Gyn. *92*, 380 (1965).
8 M.J. Whitelaw, L.R. Grams and W.J. Stamm, Am. J. Obstet. Gynec. *90*, 355 (1964).
9 O.W. Smith, G.W. Smith and R.W. Kistner, J. Am. med. Ass. *184*, 878 (1964).
10 G.M. Riley and T.N. Evans, Am. J. Obstet. Gynec. *89*, 97 (1964).
11 D. Charles, T. Klein, S.F. Lunn and J.A. Loraine, J. Obstet. Gynec. Br. Commonw. *76*, 1100 (1969).
12 S. Pandey and W.S. Hoar, Can. J. Zool. *50*, 1679 (1972).
13 D. Khosa, Thesis, Banaras Hindu University 1970.

Radioautographic demonstration of dihydrotestosterone receptor in cultured human fibroblasts

H. Ozasa, T. Tominaga, T. Nishimura and T. Takeda[1,2]

Department of Obstetrics and Gynecology, Faculty of Medicine, Kyoto University, and Department of Pathology, Chest Disease Research Institute, Kyoto University, Sakyo-ku, Kyoto 606 (Japan), 11 December 1979

Summary. Radioautographically, we obtained direct evidence for the localization of ^{3}H-dihydrotestosterone binding sites in cultured human fibroblasts derived from vulvar skin.

Evidence from biochemical studies indicates the presence of dihydrotestosterone (DHT) receptors in cultured fibroblasts derived from human skin[3]. To our knowledge, DHT receptors in fibroblasts have not been morphologically demonstrated. Using a radioautographic method developed by Weiller et al.[4], we attempted to obtain direct evidence for the intracellular localization of DHT binding sites in cultured human fibroblasts derived from vulvar skin. In the cytosols of the cells the presence of 8 S receptors was also examined by sucrose gradient analysis done in parallel studies.

Materials and methods. 1,2,4,5,6,7-^{3}H dihydrotestosterone, 123 Ci/mmole was purchased from New England Nuclear. Non-radioactive steroids were from Sigma. Nuclear emulsion (Sakura NR-M11), developer (Konidol X), and fixative (Konifix) were obtained from Konishiroku, Co.

Fibroblasts were grown from vulvar skin taken at delivery from a normal gravid woman after obtaining her consent. Cultures were maintained in a nutrient medium consisting of Eagle's minimum essential medium supplemented with 50 µg/ml of ascorbic acid and 10% fetal calf serum. Fibroblasts had undergone 5–10 passages at the time of the experiments. For 48 h preceding experiments, fibroblasts were treated with a medium containing charcoal-treated serum in place of the regular serum. Charcoal-treated serum was prepared according to the method of Griffin et al.[5].

Sucrose gradient analysis. Fibroblasts grown to confluence were collected with a rubber policeman, suspended in 2–3 ml of cold TED buffer (10 mM Tris-HCl, 1.5 mM EDTA, and 0.5 mM dithiothreitol), and homogenized in a glass-glass homogenizer. The cytosol fraction was obtained after centrifugation at 105,000 × g for 60 min and incubated with 5 nM ^{3}H-DHT with or without the addition of a 500-fold excess of unlabeled hormone for 2 h at 0–4 °C. The incubate was layered on to a linear 5–20% sucrose gradient