

and 2). Frequency of univalents was 0.12 per cell at diakinesis and 1.08 per cell at metaphase-I. At anaphase-I 32% cells showed equal distribution of chromosomes to the poles but a majority of the cells (40%) were observed with unequal distribution. As an anomaly a few cells also showed delayed separation of one bivalent (figure 4 ↑). Formation of laggards (figures 5 and 6) were recorded in 28% of cells. At sporad stage, tetrads were formed. Occasionally triads were also noticed (figure 7).

The highest number of somatic chromosomes so far reported in this species¹ is 45. Mating of an unreduced 27 chromosome gamete of the triploid with a reduced gamete of the tetraploid could have produced such a pentaploid zygote. The functioning of unreduced gametes leading to production of higher polyploid subsequent to hybridization and introgression had been demonstrated as a step towards the evolution of new chromosomal forms in *Dactylis glomerata*^{10,11}. In the present study, 56 somatic chromosomes were observed. Based on $x = 9$, the present aneuploid taxon seems to be hyper hexaploid ($2n = 6x + 2$). This constitutes the first record of the near hexaploid, aneuploid chromosome number in this species. Patil *et al*⁴ have also reported aneuploid chromosome numbers with $2n = 22$ (near diploid) and $2n = 34$ (near tetraploid) in *P. orientale*. Presence of different chromosome numbers viz 18, 22, 27, 34, 36, 45 and 56 in *P. orientale* indicates that intraspecific hybridization played a significant role in the evolution of this species. Occurrence of a high degree of polyploidy in the present material might be due to its perennial growth habit coupled with apomixis. In *P. orientale*, apomixis has been reported^{6,12}, which is of significant help in the perpetuation and stability of the aneuploids. Keeping this in view, it may be stated that the aberrant meiotic behaviour observed in the present study may not act as a barrier for perpetuation of this taxon.

27 January 1987

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IN VITRO FERTILIZATION OF RABBIT OVA

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IN VITRO fertilization (IVF) of ova could provide a large number of embryos required for the widespread application of embryo transfer (ET) for rapid multiplication of animals with the desired genetic make-up¹. However, *in vitro* fertilized rabbit ova were observed to be retarded and required to be transferred into asynchronous recipients or cultured *in vitro* for 72 hr prior to transfer into synchronous recipients². Further, studies in this area of reproductive biology are rather limited in this country. We have standardized a method for the IVF of rabbit ova which induced a minimum developmental delay.

Individually caged thirty albino rabbit does and nine bucks were utilized in this study. Capacitated sperms were flushed with one of the three fertilization media (table 1) from the uterine horns of the oestrus rabbit does mated to fertile bucks 14 hr earlier. Ovulated ova were collected³ from the fallopian tubes of the super-ovulated⁴ does around the time of ovulation. Ten to twelve ova in about 0.1 ml of fertilization medium were gently glided into 0.4 ml of capacitated sperm suspension contained in 1 ml capacity wells of a haemagglutination plate. Sperm suspensions having atleast a concentration of 10^4 sperm/ml and good whip-lash motility were only utilized in this study. The mixture of ova and sperm was covered with 0.5 ml of light weight paraffin oil equilibrated with 5% carbon dioxide in air. The plate was then transferred into an anaerobic jar containing 5% carbon dioxide in air to a pressure

Table 1 *In vitro* fertilization of rabbit ova

| Fertilization medium | A ⁷ | B ² | C* |
|--|----------------------|----------------------|----------------------|
| No. of ova put to IVF | 72 | 98 | 55 |
| No. of ova fertilized (%) | 23 (32) ^a | 35 (35) ^a | 22 (40) ^a |
| No of embryos transferred | 10 | 20 | — |
| No of embryos cultured <i>in vitro</i> | 7 | 9 | 22 |
| No of cultured embryos transferred | 7 | 7 | 18 |
| No. of bunnies born: | | | |
| Male | 2 | 1 | 1 |
| Female | 2 | 3 | 6 |

*Medium A + 25% (v/v) heat treated rabbit serum; ^aNot significantly different (χ^2 , $P < 0.01$); No bunnies were born in the case of embryos transferred.

of 3 cm of mercury⁵. The jar was then incubated at $37 \pm 0.5^\circ\text{C}$ for 24 hr. At the end of the incubation, the ova were recovered, washed in two to three changes of fresh medium to ensure removal of accessory sperm adhering to the zona pellucida and examined under a microscope. Any ova cleaved into 2 cells or more were considered fertilized at this stage and were either cultured⁶ or transferred³ into the oviducts of synchronous recipients. Some of the cultured ova were also transferred³.

Birth of bunnies subsequent to IVF and ET in this study provides the unequivocal evidence for the

Table 2 Composition of fertilization media

| Ingredient | Medium A (Brackett's medium) ⁷ (mM) | Medium B (modified Brackett's medium) ² (g/l) |
|----------------------------------|---|---|
| NaCl | 112.00 | 6.550 |
| KCl | 4.02 | 0.300 |
| CaCl ₂ | 2.25 | 0.330 |
| NaH ₂ PO ₄ | 0.83 | 0.113 |
| MgSO ₄ | — | 0.128 |
| NaHCO ₃ | 37.00 | 3.100 |
| Glucose | 13.90 | 2.500 |
| Sodium pyruvate | — | 0.055 |
| Bovine serum albumin | 3 mg/ml | 3.000 |
| Penicillin | 31 $\mu\text{g/l}$ | 1,00,000, U |
| pH | 7.8 | 7.4–7.6 |

success of IVF (table 1). All the three media tested supported IVF of rabbit ova to the same extent (table 1). The fact that *in vitro* fertilized ova developed to term in synchronous recipients only after 24 hr of *in vitro* culture indicates that the type of developmental delay observed by Seidel *et al*² occurred in this study also. But in this study much shorter period of *in vitro* culture (24 hr) was sufficient to overcome this retardation which may mean that the extent of such retardation is less or the culture system was able to overcome this delay faster or probably both.

One of the authors (VHR) thanks CSIR, New Delhi for financial assistance.

27 January 1987; Revised 5 March 1987

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STRESS-INDUCED STRUCTURAL ABERRATIONS IN THE OVARY OF IMAGINES OF *CORCYRA CEPHALONICA* (STANTON) (LEPIDOPTERA: PYRALIDAE)

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OVARIAN derangements have been noticed after treatment of juvenoids in the imagines of *Corcyra cephalonica* (Stainton)¹. In the present work an attempt has been made to compare the ovarian structural derangements induced by the topical application of three benzyloxy juvenoids AI3-63604