

PREIMPLANTATION DEVELOPMENTAL DIFFERENCES BETWEEN EMBRYO PROGENY FROM HOMOGAMETIC AND HETEROGAMETIC FEMALES IN *AKODON AZARAE* (RODENTIA, SIGMODONTINAE)

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Key words: Heterogametic females. Preimplantation embryos. XX-XY differences.

Sex in mammals is chromosomally determined through the presence of two X chromosomes in females or two different chromosomes, X and Y, in males.

Therefore, females are homogametic in that they just produce X-bearing gametes, and males are heterogametic, producing both X- or Y-bearing gametes.

Occasionally, heterogametic females (X0 or XY) appear in natural populations but they represent anomalies to the mammalian chromosome sex-determining mechanism, and display a poor fertility. Nevertheless, a few mammalian species, rodents in particular, have evolved fully fertile heterogametic females (Fredga 1988, 1994). The South American rodent *Akodon azarae* has a proportion of fertile females with sex chromosomes indistinguishable from the XY chromosomes of males, namely XY* females (Bianchi and Contreras, 1967; Espinosa and Vitullo, 1996). The remaining females show normal XX sex chromosomes.

Unexpectedly, XY* females display an enhanced reproductive capacity (Espinosa, 1995). They start to reproduce earlier, have more frequent litters that they nurse better, and stop reproduction later than XX females (Espinosa and Vitullo, 1996).

Several mechanisms have been found to be involved in maintaining heterogametism in this species. A high degree of self-synapsis of both X and Y* chromosomes at pachytene preserves the oocyte pool from functional deterioration (Solari et al., 1989). The mean ovulation number is, therefore, not significantly reduced with respect to XX females (Espinosa and Vitullo, 1996). Although YY* zygotes die early after fertilization, this does not affect litter size at birth, because XY* females ovulate more oocytes than they are able to gestate to term. The loss of YY*-embryos distorts sex-ratio to a 2 females:1 male proportion. However, a clear tendency to all-female litters in the progeny from XY* females remains to be explained (Espinosa and Vitullo, 1996).

We have investigated the effect of sex chromosome constitution on preimplantation development in embryo progeny from homogametic and heterogametic females. Mature females were induced to ovulate by the administration of 16 IU PMSG/20 IU hCG (Intervet, UK) 48 hr apart, and mated with proven fertile males. Embryos were collected at 46/48 or 72/74 hr after hCG by oviduct flushing, and classified by inspection under a stereoscopic microscope. Sex chromosome constitution of females was

known before ovulation/mating since animals are routinely karyotyped at birth by liver biopsy (Solari et al., 1989), and were confirmed by karyotyping from bone marrow cells when animals were killed for embryo collection. We analyzed the progression of development at two different time-points during the preimplantation period. The distribution of embryos was clearly different between progenies from homogametic and heterogametic females (see **Table 1**). The number of 2-cell embryos by 46-48 hr after hCG was higher for XY* mothers, increasing almost 20%. At 72-74 hr after hCG, 24% of embryos from XY* females still remained at the 2-cell stage, showing darkening and retraction of cytoplasm. This is a clear evidence that YY* embryos dye at the 2-cell stage as generally assumed for this species. By 72-74 hr after hCG, 8-cell embryos showed a significantly lower proportion in XY* female's progeny (see **Table 1**).

Compact morulae were incubated in M16 medium supplemented with colchicine for 2 hr and then processed for cell counting and karyotyping by Tarkowski's method (Tarkowski, 1966). Mean cell number in the progeny from XY* females was 23.8 ± 8.8 cells (range: 15-38 cells). This value was two-fold higher than the mean cell number for XX female's progeny (11.7 ± 3.9 cells, range: 8-20); this difference was statistically significant (*t*-test, $p < 0.01$). Twelve morulae that were accurately karyotyped as being from XY* progeny, re-

sulted in 9 XY and 3 XX embryos. For XX progeny, 19 accurately karyotyped morulae resulted in 8 XY and 11 XX embryos. XY embryos from XY* females were mostly observed among the progeny with higher cell numbers, while those from XX females were distributed throughout the whole observed range (data not shown).

A preliminary analysis at a later time-point (90 hr post-hCG injection) showed greater proportion of fully expanded blastocyst in the progeny of XY* females (85%, $N=20$) vs. 50% ($N=12$) rate in XX females.

These results show that embryo progeny develops faster in XY* females and that this acceleration seems to be related to the presence of the Y* chromosome. Differences between the sexes before the development of the gonads have been described in several mammalian species (Renfree and Short, 1988). In the laboratory mouse, it has been shown that the Y chromosome of the CD1 strain has an accelerating effect on preimplantation development (Burgoyne, 1993). As suggested by A. McLaren (in Burgoyne, 1993), this may favour XY embryos at the time of implantation. In this scenario, self-synapsis of sex chromosomes, unaltered ovulation rate, and the accelerated preimplantation development of XY* embryos can act together to successfully maintain this exceptional condition in natural populations of *Akodon azarae* by enhancing the chances of implantation of XY female embryos.

Table 1

Differential preimplantation developmental rate in embryo progeny from heterogametic (XY*) and homogametic (XX) females of *Akodon azarae*.

hr post hCG	Female karyotype (No. females)	Number of embryos (%)				
		2-cell	4-cell	8-cell	morulae	total
46-48	XY*(3)	25 (80.6) ¹	6 (19.3)			31
	XX (4)	18 (62.0) ¹	11 (37.9)			29
72-74	XY*(5)	10 (23.8)	3 (7.1)	1 (2.4)	28 (66.7)	42
	XX (3)	4 (7.4)	2 (3.7)	6 (11.2)	42 (77.8)	54

¹ The proportion of 2-cell embryos was significantly different (χ^2 test, $p < 0.05$)

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