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Fast-developing preimplantation embryo progeny from heterogametic females in mammals

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Summary

Karyotyping and cell number estimates in preimplantation embryos from heterogametic (XY*) and homogametic (XX) females of the field mouse *Akodon azarae* were studied to determine whether XX–XY–XY* differences exist in the rate of preimplantation development. At the morula stage, XY embryos from heterogametic mothers had twice the mean number of cells compared with XX embryos. However, this difference in cell numbers was not seen between XX and XY embryos from homogametic mothers. In this case, mean cell numbers were similar despite embryos being XX or XY. Furthermore, the mean cell number for XX and XY morulae from homogametic females was comparable to that for XX embryos from heterogametic females. It is concluded that XY* embryos (which will develop into heterogametic females) show an accelerated rate of preimplantation development.

Keywords: Heterogametism, XX–XY differences, XY females, Y chromosome effect

Introduction

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 produce only X-bearing gametes, and males are heterogametic, producing both X- or Y-bearing gametes. Occasionally, heterogametic females (XO or XY) appear in natural populations but they represent anomalies to the mammalian chromosome sex-determining mechanism, and display a poor fertility (Fredga, 1988). Nevertheless, a few mammalian species, rodents in particular, have evolved fully fertile heterogametic females (Fredga, 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 & Contreras, 1967; Espinosa & Vitullo, 1996). The remaining females show normal XX sex chromosomes. Unexpectedly, XY* females display an enhanced reproductive capacity. They start to reproduce earlier, have more frequent litters that they nurse better, and stop reproduction later than XX females (Espinosa & 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). Mean ovulation number is, thus, not significantly decreased with respect to XX females (Espinosa & Vitullo, 1996). Moreover, although YY* zygotes die early after fertilisation, it does not affect litter size at birth since XY* females ovulate more oocytes than the number of embryos they are able to gestate to term. YY* embryo loss distorts the sex ratio to a female male proportion of 2:1. However, a clear tendency to all-female litters in the progeny from XY* females remains to be explained (Espinosa & Vitullo, 1996). It has been shown for the mouse strains CD1 and MF1 that the Y chromosome has a factor that accelerates the rate of preimplantation development (Burgoyne, 1993). This situation may favour the implantation of XY embryos, so that more XY than XX embryos implant (A. McLaren in Burgoyne, 1993).

We have investigated the effect of sex chromosome constitution on preimplantation development in embryo progeny from homogametic and heterogametic females of *Akodon azarae*. We find that there is a

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difference in preimplantation developmental rate, XY* embryos being more advanced than XX (and even XY) individuals at the same gestational age.

Materials and methods

Animals

Akodon azarae individuals used in this study came from our own laboratory colony established in 1982. The animals were maintained under standard conditions (20 ± 2 °C; 12 h dark/12 h light; water and food *ad libitum*). Detailed information on husbandry, postnatal development and general conditions for reproduction have been published elsewhere (Espinosa, 1995).

Embryo recovery

Mature females were induced to ovulate by intraperitoneal injection of 16 IU of pregnant mare's serum gonadotropin (PMSG; Folligon, Intervet) followed 72 h later by 20 IU of human chorionic gonadotropin (hCG; Chorulon, Intervet). PMSG was given to females regardless of the stage of the oestrous cycle. Immediately after hCG administration, females were mated with proven fertile males in monogamous pairs. Males were removed the next morning and females were kept individually until used for embryo recovery at 46–48 or 72–74 h after hCG. Sex chromosomes of females were known before ovulation/mating since animals are routinely karyotyped at birth by liver biopsy (Solari *et al.*, 1989). However, 1.5 h before collecting embryos, females were intraperitoneally injected with colchicine (1 µg/g body weight). After that, females were killed by cervical dislocation, sex chromosomes (XX or XY*) were confirmed by karyotyping from bone marrow cells by standard techniques, and the oviducts were removed and flushed with M2 medium for embryo recovery.

Preimplantation embryo preparation

Embryo progenies were inspected for viability and cell number estimation by means of a stereoscopic microscope, placed in drops of M16 medium containing 0.1 µg/ml of colchicine, and incubated under paraffin oil at 37 °C in an atmosphere of 5% CO_2 in air for 1.5–2.5 h. After incubation, embryos were processed individually according to Tarkowski (1966) for chromosome preparation. In brief, the embryo was placed in 1% trisodium citrate for 2.5–3 min. It was then transferred in a fine glass pipette to a clean slide, and two or three drops of freshly prepared cold fixative (3:1 methanol:acetic acid) added while observing the embryo under a dissection microscope. The preparation was then blown dry and stained in 2% Giemsa. Cell counts were performed under a ×16 objective, and metaphases were analysed under a ×100 oil immersion objective. Scoring for the presence of a Y chromosome was simple since the Y chromosome is similar in length to autosomal pair 16, the smallest pair of the complement. Thus XY or XY* cells display three small chromosomes, while XX cells show just two small chromosomes (pair 16). In addition, the number of X chromosomes can easily be assessed since the X is the only large chromosome with short arms (Fig. 1).

Results

The progression of development at two different timepoints during the preimplantation period showed

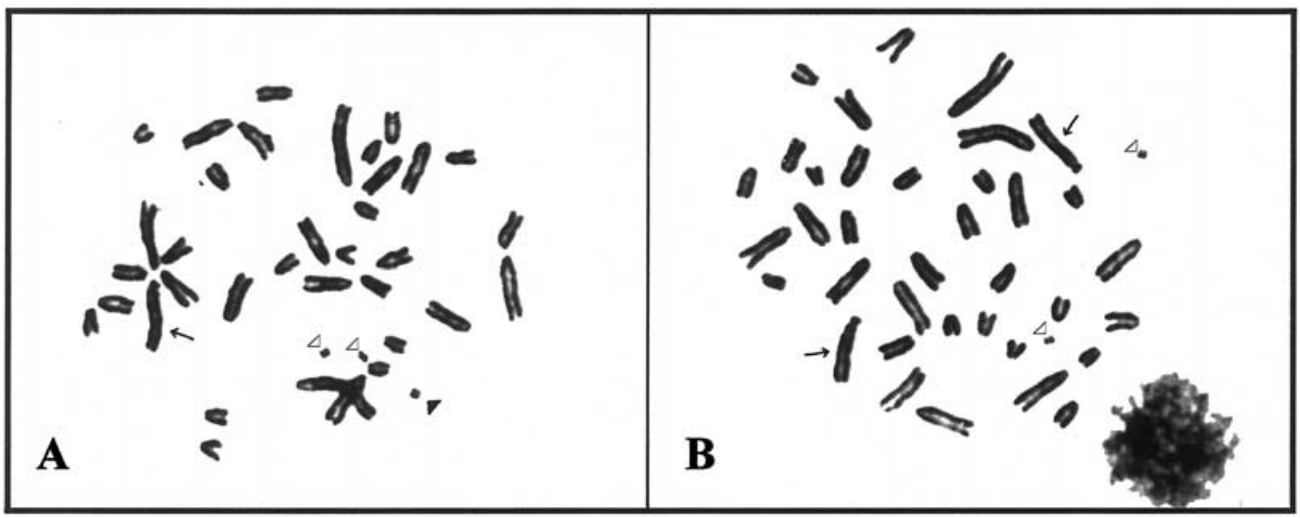

Figure 1 Chromosome preparations from preimplantation embryos of *Akodon azarae* (2n=38). (*A*) Metaphase (×1000) of an XY (or XY*) embryo; (*B*) metaphase from an XX embryo. Arrows show X chromosomes, the filled arrowhead indicates the Y (or Y*) chromosome, and open arrowheads show autosome pair 16. For details see text.

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some relevant differences between progenies from homogametic and heterogametic females. The number of 2-cell embryos by 46–48 h after hCG was significantly higher for XY* mothers, being increased by almost 25% (Table 1). By 72–74 h after hCG, 23.8% of embryos from XY* females still remained at the 2-cell stage showing darkening and retraction of cytoplasm. This figure correlates very well with the expected 25% embryo loss when XY* females are mated to XY males, and is clear evidence that YY* embryos die at the 2-cell stage as generally assumed for this species.

While development seems to be retarded in XY* progeny at the 2-cell/4-cell transition in comparison with homogametic females, this situation reverses at 72–74 h (Table 1). The number of 8-cell embryos at 72–74 h after hCG was significantly misrepresented in the progeny of XY* females. The percentage of morulae is not significantly different for the two female types. This is particularly evident if the numbers in row three of Table 1 are corrected by excluding 2-cell embryos showing clear signs of developmental arrest and death. In that situation the corrected number of morulae for XY* females reaches 80%.

Compact morulae were incubated in M16 medium supplemented with colchicine for 2 h and then processed for cell counting and karyotyping by Tarkowski's method. Mean cell number in the progeny from XY* females was 23.8 ± 8.8 cells (range 15–38 cells). This value was twofold higher than the mean cell number for the progeny of XX females (11.7 ± 3.9 cells, range 8–20 cells).

Of 28 morulae from XY* mothers processed for cell counting, 11 lacked metaphases. Of the 17 morulae with metaphases, 12 (70%) provided chromosome counts and accurate identification of sex chromosomes. Of these accurately karyotyped 12 embryos, 9 were XY (or XY*) and 3 were XX. For XX mothers, 18 of 42 morulae processed lacked metaphases. Of the 24 morulae with metaphases, 19 (79%) provided chromosome counts and accurate identification of sex chromosomes, resulting in 8 XY and 11 XX embryos.

XY embryos from XY* females were mostly associated with the highest cell numbers within the range, while XX embryos were associated with low cell numbers (Table 2). On the contrary, embryos from XX females displayed a similar distribution of cell numbers irrespective of being XX or XY (Table 2). It is worth noting that some XY embryos from XY* mothers showed a low cell count, comparable to cell counts recorded for XX embryos (see Table 2, embryos with 18 and 20 cells) in the same progeny. Moreover, these cell counts are in the same range found for both XX and XY embryos from XX mothers.

Discussion

The results presented here demonstrate that XX, XY and XY* embryos of *Akodon azarae* show different rates of preimplantation development, XY* individuals being developmentally more advanced than XX, and even XY, embryos. Embryo progeny from heterogametic (XY*)

Table 1 Differential preimplantation development rate in embryo progeny from heterogametic (XY*) and homogametic (XX) females of *Akodon azarae*

Time after hCG (h)	Female karyotype	No. of embryos (%)				
		2-cell	4-cell	8-cell	Morulae	Total
46–48	XY*	25 (80.6)	6 (19.3)			31
	XX	18 (62.0)	11 (37.9)			29
72–74	XY*	10 (23.8)	3 (7.1)	1 (2.4)	28 (66.7)	42
	XX	4 (7.4)	2 (3.7)	6 (11.2)	42 (77.8)	54

Table 2 Cell number in embryos from heterogametic and homogametic females of *Akodon azarae* in relation to sex chromosomes

Female type	No. of embryos	Embryo karyotype	Cell number[a]
XY*	9	XY (or XY*)[b]	38, 34, 27, 18, 28, 36, 30, 28, 20,
	3	XX	18, 14, 16
XX	8	XY	18, 20, 16, 17, 20, 14, 12, 17,
	11	XX	12, 14, 17, 16, 12, 15, 12, 18, 10, 12, 11

[a] Morulae were placed in colchicine-containing medium immediately after collection. The cell number therefore represents the number of cells at collection time (72–74 h after hCG).
[b] Y and Y* chromosomes are cytogenetically indistinguishable.

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females show a mean cell number at the morula stage which is twofold higher than that of embryo progeny from homogametic females. This increase seems to be related to the presence of the Y* chromosome. The distribution of cell numbers for individual embryos is comparable for XX morulae from both XX and XY* mothers. Moreover, XY morulae from XX mothers do not show differences in cell numbers when compared with XX morulae. However, cell numbers in XY morulae from XY* mothers are higher, at least for most of them (Table 2). This strongly suggests that the increase in mean cell number reflects an accelerated rate of development that is especially attributable to XY* embryos, being therefore 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. It has been reported for mice and cattle that XY embryos are more advanced than XX embryos during preimplantation development (Tsunoda *et al.*, 1985; Gardner & Lesse, 1987; Avery *et al.*, 1991; Xu *et al.*, 1992). In the laboratory mouse, Burgoyne (1993) has shown that the Y chromosome of the CD1 and MF1 strains has an accelerating effect on preimplantation development that is probably due to a Y gene with a positive effect on the rate of cell division. In *A. azarae* it is worth considering that the Y* chromosome responsible for the production of XY* females is strictly inherited and maintained through the heterogametic female lineage. There may therefore have been selection in favour of factors that increase the rate of preimplantation development.

Dr A. McLaren (in Burgoyne, 1993) has suggested that the accelerated rate of preimplantation development may favour XY embryos at the time of implantation, so that more XY than XX embryos implant. This hypothesis, together with the results presented here, may explain why heterogametic *A. azarae* females show a bias towards all-female litters (Espinosa & Vitullo, 1996).

The accelerated rate of preimplantation development of XY* embryos provides new insights into how female heterogametism is efficiently maintained in *A. azarae*. This maintenance arises from the conjunction of different mechanisms. First, X and Y* chromosomes show a high rate (~60%) of self-synapsis during the pachytene stage of meiosis of heterogametic females (Solari *et al.*, 1989). Self-synapsis enables the oocytes to escape from functional deterioration, since unsynapsed sex chromosomes in heterogametic mice are known to seriously affect the size of the postnatal oocyte pool (Burgoyne *et al.*, 1992). Second, an automatic process of reproductive compensation for the loss of YY* zygotes has been described (Espinosa & Vitullo, 1996). XY* females ovulate more oocytes than the number of embryos they are able to gestate to term. Finally, XY* embryos have increased chances of implantation since they develop faster during the preimplantation period, as shown in this report.

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References

Avery, B., Madison, V. & Greve, R. (1991). Sex and development in bovine *in-vitro* fertilised embryos. *Theriogenology* **35**, 953–63.

Bianchi, N.O. & Contreras, J.R. (1967). The chromosomes of the field mouse *Akodon azarae* (Cricetidae–Rodentia) with special reference to sex chromosome anomalies. *Cytogenetics* **6**, 306–13.

Burgoyne, P.S. (1993). A Y-chromosomal effect on blastocyst cell number in mice. *Development* **117**, 341–5.

Burgoyne, P.S., Tam, P.P.L. & Evans, E.P. (1983). Retarded development of XO conceptuses during early pregnancy in the mouse. *J. Reprod. Fertil.* **68**, 387–93.

Burgoyne, P.S., Mahadevaiah, S.K., Sutcliffe, M.J. & Palmer, S.J. (1992). Fertility in mice requires X–Y pairing and a Y-chromosomal spermiogenesis gene mapping to the long arm. *Cell* **71**, 391–8.

Espinosa, M.B. (1991). Hembras hetero y homogaméticas en el roedor cricétido *Akodon azarae*: su desempeño reproductivo y mantenimiento en condiciones de laboratorio. PhD dissertation, University of Buenos Aires, 139pp.

Espinosa, M.B. (1995). *Akodon azarae* (Rodentia, Cricetidae): breeding, management and reproductive performance in laboratory conditions. *Braz. J. Biol.* **55**, 201–6.

Espinosa, M.B. & Vitullo, A.D. (1996). Offspring sex-ratio and reproductive performance in heterogametic females of the South American field mouse *Akodon azarae. Hereditas* **124**, 57–62.

Fredga, K. (1988). Aberrant chromosomal sex-determining mechanisms in mammals, with special reference to species with XY females. *Phil. Trans. R. Soc. Lond. B* **322**, 83–95.

Fredga, K. (1994). Bizarre mammalian sex-determining mechanisms. In *The Differences Between the Sexes* (ed. R.V. Short & E. Balaban), pp. 419–31. Cambridge: Cambridge University Press.

Gardner, D.K. & Leese, H.J. (1987). Assessment of embryo viability prior to transfer by the non-invasive measurement of glucose uptake. *J. Exp. Zool.* **242**, 103–5.

Solari, A.J., Espinosa, M.B., Vitullo, A.D. & Merani, M.S. (1989). Meiotic behaviour of gonosomically variant females of *Akodon azarae* (Rodentia, Cricetidae). *Cytogenet. Cell Genet.* **52**, 57–61.

Tarkowski, A. (1966). An air-drying method for chromosome preparations in mouse embryos. *Cytogenetics* **5**, 394–6.

Tsunoda, Y., Tokunaga, T. & Sugie, T. (1985). Altered sex-ratio of live young after transfer of fast- and slow-developing mouse embryos. *Gamete Res.* **12**, 301–4.

Xu, K.P., Yadav, B.R., King, W.A. & Betteridge, K.J. (1992). Sex-related differences in developmental rates of bovine embryos produced and cultured *in vitro*. *Mol. Reprod. Dev.* **31**, 249–52.