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Conservation of Houbara bustards using interspecific chimeras

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ABSTRACT

Embryonic gonadal tissue was dissected individually from Houbara bustard embryos at eight days post-incubation. Houbara bustard gonadal cells containing gonadal primordial germ cells (gPGCs) were injected into the blood stream of White Leghorn chicken (*Gallus gallus domesticus*) embryos, producing 83/138 surviving male chimeric embryos, of which 35 chimeric roosters reached sexual maturity after 5 months. The incorporation and differentiation of Houbara bustard PGCs in chimeric chicken testis were assessed by PCR with Houbara bustard species-specific primers and 31.3% (5/16) gonads collected from the injected chicken embryos showed the presence of donor Houbara bustard cells. A total of 302 semen samples from 34 chimeric roosters were analyzed and eight were confirmed as germ line chimeras. Semen samples from these eight roosters were used to artificially inseminate three female Houbara bustards. Subsequently, 45 Houbara bustard eggs were obtained and incubated, two of which were fertile. One egg hatched as a male live born Houbara bustard and the other was female but died before hatching.

Keywords: *Houbara bustard (*Chlamydotis undulata*), primordial germ cells (PGCs), interspecies germ line chimera, chicken*

1. INTRODUCTION

The Houbara bustards (*Chlamydotis undulata undulata*) belong to the order Gruiformes, and is classified as vulnerable. It breeds in deserts and other very arid sandy areas and is largely resident within its range. The domesticated chicken (*Gallus gallus domesticus*) belongs to the order *Galliformes*. A major priority for Houbara bustard breeding conservationists is to generate a means by which Houbara bustards could be produced with the fecundity of chickens. Primordial germ cell-mediated chimera technology is a promising approach with the potential to achieve this.

Avian primordial germ cells (PGCs), precursor of the germ cells, are epiblastic origin (Eyal-Giladi *et al.*, 1981) and it migrate to germinal crescent region during the first two days of development. Unlike mammalian PGCs, the avian PGCs migrate through the blood circulation to the developing embryonic gonad. Circulating PGCs (Tajima *et al.*, 1993) and gonadal PGCs (gPGCs, Chang *et al.*, 1995, 1997) can be transferred into another chicken embryonic blood circulating system and can contribute a chimeric germ line, which produces functional gametes. Based on intra-species, inter-species chimera derived progenies have been produced Duck (*Anus platrhynchos*) and chicken using blastoderm cell transfer (Li *et al.*, 2002) and between pheasant (*Phasianus colchicus*) and chicken (Kang *et al.*, 2008). The present study was undertaken to determine whether Houbara bustard gPGCs could produce functional gametes when in a chicken background.

2. MATERIALS AND METHODS

Houbara bustards were raised and bred in the Houbara Breeding Center of the Central Veterinary Research Laboratory (CVRL), Dubai, UAE. White Leghorn chickens were maintained in the Chicken House of CVRL. Fertilized fresh Houbara bustard eggs were collected and incubated for 8 days at 37.8°C and 60% relative humidity. The gonadal tissue was collected individually from Houbara bustard embryos under the stereomicroscope according to Chang *et al.* (1995), and single cell suspension was adjusted to 4×10^6 cells per mL in DMEM before transfer.

Fertilized White Leghorn chicken eggs were incubated for 2.5 days until embryonic stage 15–16 (Hamburger and Hamilton, 1951) at 37.8°C and at

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60% relative humidity. A small window (about 10 mm in diameter) was made into the shell on the sharp end. A total number of 8,000 Houbara bustard gonadal cells were injected into the dorsal aorta of chicken embryos using a fine glass pipette. The injected eggs were sealed with a piece of parafilm and incubated to hatch with the blunt end up. Gonadal tissues were collected from the embryos that died during the week before hatch. The hatched chicks were raised to sexual maturity. Semen samples were collected from 34 chimeras weekly for a period of three months. Freshly collected semen was diluted in PBS(-) and 50 µL of sample was used to detect the existence of Houbara bustard sperm by a PCR species identification test as described (Wernery *et al.*, 2010). During the breeding season between January and May, semen samples were collected from eight male chimeric roosters that had produced Houbara bustard-DNA positive semen.

Sex identification was performed according to Ogawa *et al.* (1997), primers USP1 and USP3 were used to determine the sex, Myo INT1 and Myo INT2 were used as internal control primers (Wernery *et al.*, 2010). The specific primers CHN1F and CHN1R (Pitra *et al.*, 2000), BTD2F and BTD2R (Ogawa *et al.*, 1997) were used for identification of chicken and Houbara bustard species, respectively. The primers for Houbara bustard species identification are designed from the cytochrome b gene found in the mitochondria (Pitra *et al.*, 2000).

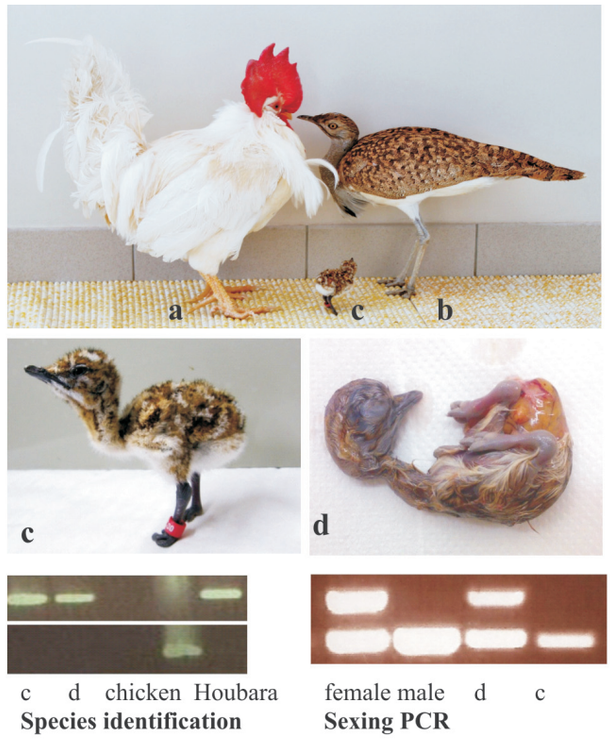

Figure 1 Houbara-chicken chimeric rooster (**a**) and pure female Houbara bustard (**b**) produced pure Houbara progenies (**c** and **d**). Both of the live chick (**c**, male) and the dead embryo (**d**, female) are proven to be pure Houbara bustard by species identification PCR.

3. RESULTS

The total number of gonadal cells in 8-day-old Houbara bustard embryos was 114,800 ($\pm$ 20.5, $n = 23$) and 102,700 ($\pm$ 21.2, $n = 15$) in male and female embryos, respectively. The morphological characteristics of Houbara bustard gPGCs were similar to that of chicken. They can be mostly distinguished from somatic cells by being rounder and larger in size (12–15 µm in diameter) as well as rich in granules in the cytoplasm than somatic cells. A total number of 138 chicken embryos were injected with Houbara bustard gonadal cells from individual male embryos, out of which 83 (60.1%) hatched. A total number of 35 male and 35 female reached sexual maturity after 5 months.

Houbara bustard-specific DNA fragments were amplified from 31.3% (5/16) gonadal tissues of the injected chicken embryos. A total of 302 semen samples were collected from 34 chimeric roosters. Houbara bustard species-specific DNA fragments were amplified from the semen samples of 23.5% (8/34) roosters. Furthermore a total number of 95 semen samples were collected from these eight germ line chimeric roosters, out of which 9.5% (9/95) were confirmed as Houbara bustard-DNA positive. These results suggested that Houbara bustard gonadal cells injected in the early embryonic stage are able to migrate into the recipient gonads and differentiate into sperm in the testis of chimeric rooster.

Three female Houbara bustards (Figure 1b) were inseminated 198 times from these eight germ line chimeric roosters (Figure 1a). A total number of 45 Houbara bustard eggs were obtained and incubated, of which two eggs were found to be fertile. One successfully hatched after 22 days of incubation (Figure 1c), while the other fully developed embryo died just before hatching (Figure 1d). The chick and the dead embryo showed typical Houbara bustard phenotype, albeit with minor deformity in the toes of both feet. The chick was identified by PCR as male; the dead embryo was female (Figure 1).

4. DISCUSSION

In the present study, a viable Houbara bustard was produced following a mating of a male chimeric rooster and pure female Houbara bustard for the first

time. This study demonstrates that Houbara bustard PGCs are able to migrate into the chicken recipient gonads and differentiate into functional sperm. The chimeric rooster thus served as a surrogate father of the chick, suggesting that the reproduction organs, including hormonal systems, might be conserved in different avian species or orders.

It is reported that pheasant PGCs derived progenies were produced from chimeric rooster (Kang *et al.*, 2008). Also donor DNA was detected in semen sample from chicken-quail chimera; but no progeny was obtained (Li *et al.*, 2001). Highly sensitive molecular sexing and species identification PCR methods for Houbara bustard cells were developed as a result of the present research, also providing strong molecular tools for Houbara bustard research.

The Houbara bustard reaches sexual maturity in about two years (Saint-Jalme and Heezik, 1995). In the present study, Houbara bustard sperm could be detected when the chimeric rooster reached sexual maturity in about five months, indicating that donor spermatogenesis occurs in same time that the recipient spermatozoa form. It is also confirmed that the chimeric roosters produce Houbara bustard sperm for up to three years. The donor Houbara bustard DNA was not detected in weekly semen sample, but no specific pattern was observed. Furthermore, Houbara bustard DNA was detected in semen during the off-breeding season. It is also not clear if the donor sperm was generated and released in the host testis by its own spermatogenesis process. These observations suggest that the Houbara bustard PGCs follow the non-seasonal breeding reproduction pattern similar to that in chicken. This will increase the chance to produce even more Houbara bustards using the domestic male chicken reproduction system all year round.

The efficiency of the progeny production is still low. It is important to note that both were identified as pure Houbara bustard, not hybrid, and not from a parthenogenic development (data not shown). Furthermore, if female PGCs could differentiate into functional ova in the ovary of chimeric hen, mating the male and female chimeric chicken could reproduce Houbara bustards in the future, and this will greatly increase the Houbara bustard population using the chimeric technology. In the end, this technology could also be applied to the conservation of other endangered avian species that cannot be bred in captivity.

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REFERENCES

Chang, I.K., Done, K.J., Hong, Y.H., Park, T.S., Moon, Y.K., Ohno, T. and Han, J.Y. (1997) Production of germ line chimeric chickens by transfer of cultured primordial germ cells. *Cell Biol. Int.*, **21**, 495–499.

Chang, I.K., Yoshiki, A., Kusakabe, M., Tajima, A., Chikamune, T., Naito, M. and Ohno, T. (1995) Germ line chimera produced by transfer of cultured chick primordial germ cells. *Cell Biol. Int.*, **19**, 569–576.

Eyal-Giladi, H., Ginsburg, M. and Farbarov, A. (1981) Avian primordial germ cells are of epiblast origin. *J. Embryo Exp. Morphol.*, **63**, 139–147.

Hamburger, V. and Hamilton, H.L. (1951) A series of normal stage in the development of the chick embryo. *J. Morphol.*, **88**, 49–92.

Kang, S.J., Choi, J.W., Kim, S.Y., Park, K.J., Kim, T.M., Lee, Y.M., Kim, H., Lim, J.M. and Han, J.Y. (2008) Reproduction of wild birds via interspecies germ cell transplantation. *Biol. Reprod.*, **79**, 931–937.

Li, H.C., Zhao, L., Kagami, H., Matsui, K. and Ono, T. (2001) Identification of transferred chicken germ cells in quail gonad and semen by amplification of chicken-specific PCR products. *J. Poult. Sci.*, **38**, 308–316.

Li, Z.D., Deng, H., Liu, C.H., Song, Y.H., Sha, J., Wang, N. and Wei, H. (2002) Production of Duck-chicken chimeras by transferring early blastoderm cells. *Poult. Sci.*, **81**, 1360–1364.

Ogawa, A., Solovei, I., Hutchison, N., Saitoh, Y., Ikeda, J.E., Macgregor, H. and Mizuno, S. (1997) Molecular characterization and cytological mapping of a non-repetitive DNA sequence region from the W chromosome of chicken and its use as a universal probe for sexing carinatae birds. *Chromosome Res.*, **5**, 93–101.

Pitra, C., Lieckfedt, D. and Alonso, J.C. (2000) Population subdivision in Europe's great bustard inferred from mitochondrial and nuclear DNA sequence variation. *Mol. Ecol.*, **9**, 1165–1170.

Saint-Jalme, M. and Van Heezik, Y. (1995) *Propagation of the Houbara Bustard*. London: Keegan Paul International, pp. 70–96.

Tajima, A., Naito, M., Yasuda, Y. and Kuwana, T. (1993) Production of germ line chimera by transfer of primordial germ cells in the domestic chicken (*Gallus domesticus*). *Theriogenology*, **40**, 509–519.

Wernery, U., Liu, C.H., Baskar, V., Guerineche, Z., Khazanehdari, K., Saleem, S., Kinne, K., Wernery, R., Griffin, D.K. and Chang, I.K. (2010) Primordial germ cell-mediated chimera technology produces viable pure-line Houbara bustard offspring: Potential for repopulating an endangered species. *PLoS ONE*, **5**(12), \e15824.