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An Attempt at Hybridization of Farmed Axis (*Axis Axis*) and Fallow Deer (*Dama Dama*) by Intrauterine Laparoscopic Artificial Insemination

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Abstract: A study was conducted to determine whether axis (*Axis axis*) and fallow deer (*Dama dama*) could be hybridized using artificial insemination (AI). The feasibility of hybridization between these two deer species has implications for free-ranging (mixed) populations of deer species and intensive deer farming production systems. An intravaginal progesterone-releasing device (CIDR) was inserted into axis (n=16) and fallow (n=38) does for 14 d to synchronize estrus. Following CIDR removal, axis does were artificially inseminated laparoscopically with fallow buck semen and fallow does with axis buck semen. Blood samples were collected on d 21, 28, 35 and 42 post-AI for determination of serum concentrations of progesterone (P_4) and serum pregnancy-specific protein B (PSPB) measured on d 28 and 35 post-AI. Transrectal ultrasonography was performed on d 42 post-AI for pregnancy detection. On d 14 (axis does) or 17 (fallow does) post-AI, axis bucks and fallow bucks, respectively, were placed with same-species does for 60 d and subsequent pregnancy and fawning data recorded. Ultrasonography revealed that none of the axis or fallow does conceived to the inter-species AI. Serum concentrations of P_4 were not elevated 21 d post-AI and serum PSPB was undetectable at d 28 and 35 post-AI for all does. Axis and fallow does maintained with same species axis and fallow clean up sires post-AI resulted in pregnancy rates of 93.8 and 89.5%, respectively and fawning rates of 72.7 and 87.5%, respectively. Using laparoscopic AI procedures, hybridization of axis and fallow does inseminated with fallow and axis buck semen, respectively, did not result in apparent pregnancies. Given the genetic differences between axis and fallow deer (chromosome number: 2n=66 and 68, respectively) and the absence of documented reports of hybridization occurring in large free ranging populations of axis and fallow deer, hybridization between these two species appears unlikely and requires further study.

Key words: Axis deer, fallow deer, hybridization, artificial insemination

INTRODUCTION

Cross-breeding within genus among closely related breeds (and in some cases, species), is a common practice in traditional livestock production systems (e.g. *Bos indicus* and *Bos taurus* cattle), while interspecies hybridization of more distantly related cousins often results in sterile offspring (e.g. *Equus asinus* or *Equus zebra* and *Equus caballus*). Within the genus *Cervus*, hybridization among wapiti (*Cervus elaphus* spp.), red deer (*Cervus elaphus*) and sika deer (*Cervus nippon* spp.) has occurred naturally in some regions and is a breeding practice used for increasing growth rates of offspring and antler weights of stags in some deer farming systems[1-8]. Hybridization of Pere David's deer (*Elaphurus davidianus*) with red deer has also been achieved as a means for producing rapidly growing calves and advancing the breeding season, however this species is considered to be more distantly related to red deer than wapiti and sika deer[5,9]. While red deer and Pere David's deer have the same chromosome number (2n=68), there have been reports of a lower success rate in producing F_1 hybrids, with pregnancy rates of only 13% and subsequent calving rates of 60%[9].

Fallow deer (*Dama dama*) and axis deer (*Axis axis*) differ with regards to reported numbers of chromosomes for these two species; 2n=68 and 66, respectively[10-12]. In addition, listings of reported hybridization (anecdotal or otherwise) among various deer species do not include documented reports of axis and fallow deer hybridizing[12-13]. However, axis deer will reciprocally cross with hog deer *Axis poreins*[12,13] and a report of a hog deer buck crossing with a fallow doe resulting in a live offspring was reported in 1958[12]. It should be noted that hog deer have a reported chromosome number of 2n= 68 which is the same as fallow deer, yet hog deer and axis

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deer appear to cross more readily as indicated in documented reports of such breedings[12,13]. Hybridization between a sika deer (2n=68) and axis deer (2n=66) has also been reported[14], which would further suggest the possibility that an axis x fallow hybridization might also be feasible. Considering the numbers of introduced axis and fallow deer currently free-ranging in mixed populations in some regions (e.g. Texas: > 51,000 axis deer and 20,000 fallow deer;[15], information regarding the ability of these two species to hybridize is of interest. While the absence of reports of axis x fallow deer hybrids in free-ranging or captive environments would suggest hybridization between these two species is unlikely, the potential implications of interspecies hybridization and the ramifications with regard to genetic preservation is cause for investigation. Moreover, from a deer farming or production perspective, crossbred offspring with the temperament and adaptability of fallow deer and the non-seasonal breeding and venison quality of axis deer from F_1 or composite hybrids could be extremely beneficial in some deer farming or game ranching systems.

To this end, an experiment was conducted to reciprocally cross axis and fallow deer using standard laparoscopic artificial insemination procedures to determine whether a hybrid could be created in this manner.

MATERIALS AND METHODS

Artificial insemination of axis does with frozen-thawed fallow deer semen: An intravaginal progesterone-releasing device (CIDR type-G; Pharmacia and Upjohn, Rydalmere, NSW) was inserted into each axis doe (n=16; body weight: 46.0±0.9 kg) for 14 d to synchronize estrus. Following CIDR removal, does were artificially inseminated at 67.2±0.2 h post-CIDR removal with fallow deer semen using standard laparoscopic artificial insemination procedures[16]. Frozen-thawed extended semen used for the AI of axis does was collected from one of two fallow bucks. Twelve axis does were inseminated with semen from fallow buck-1 (Buck ID: Fallow Spot No.2), using a total dose of 41.6 x 10^6 spermatozoa per 0.25 c c straw and an average post-thaw progressive motility of 40% and the remaining n=4 does were inseminated with semen from fallow buck-2 (ID: Fallow White No.1), using a total dose of 77.0 x 10^6 spermatozoa per 0.25 c c straw and an average post-thaw progressive motility of 50%. Intrauterine AI performed laparoscopically was accomplished following i.v. anaesthesia of axis does with xylazine hydrochloride (1.9±0.1 mg kg^{-1}; Rompun; Miles Inc., Shawnee Mission, KS) and ketamine hydrochloride (2.7±0.1 mg kg^{-1}; Ketaset; Aveco Co., Inc., Fort Dodge, IA). Does were inseminated directly into the lumen of the uterine horns, splitting the 0.25 cc dose evenly between the right and left uterine horns. Following laparoscopic AI, anaesthesia was reversed by yohimbine hydrochloride (0.10±0.01 mg kg^{-1}; Yobine; Lloyd Laboratory, Shenandoah, IA) and each doe received 300,000 U of penicillin i.m. (1 cc; Microcillin; Pacific Animal Health, Arcadia, CA). Fourteen days post-AI, two axis bucks were placed with the herd of axis does for a 60-d breeding season. Blood samples were collected from does on d 21, 28, 35 and 42 post-AI for the collection of serum which was stored at -20°C until analysis for concentrations of P_4 by RIA[17,18]. Serum Pregnancy-specific Protein B (PSPB) was also determined from serum samples taken on d 28 and 35 post-AI by RIA[17,18]. Transrectal ultrasonography (5 Mhz transducer; Aloka 210 ultrasound, Corometrics Medical Systems, Inc., Wallingford, CT) was performed on d 42 post-AI to determine pregnancy rate from AI and was repeated at 35 d after buck removal following the 60-d natural breeding period. Subsequent doe fawning data was also collected.

Artificial insemination of fallow does with frozen-thawed axis deer semen: A CIDR was inserted into each fallow doe (n=38; body weight: 41.5±0.7 kg) for 14 d to synchronize estrus. Following CIDR removal, does were artificially inseminated at 64.8±0.2 h post-CIDR removal with axis deer semen using standard laparoscopic artificial insemination procedures as described previously. Frozen-thawed extended semen used for the AI of fallow does was collected from one axis buck (ID: Delbert). An average total dose of 98.0 x 10^6 spermatozoa per 0.25 c c straw was used for each insemination with an average post-thaw progressive motility of 71.7±1.5%. Intrauterine AI performed laparoscopically was accomplished following i.v. anaesthesia of fallow does with xylazine hydrochloride (1.0±0.03 mg kg^{-1}) and ketamine hydrochloride (2.0±0.1 mg kg^{-1}). Does were inseminated directly into the lumen of the uterine horns, splitting the 0.25 c c dose evenly between the right and left uterine horns. Following laparoscopic AI, anaesthesia was reversed by yohimbine hydrochloride (0.20±0.01 mg kg^{-1}) and each doe received 300,000 U of penicillin i.m. Seventeen days post-AI, three fallow bucks were placed with the herd of fallow does for a 60-d breeding season. Protocols for blood sampling, hormone analysis, ultrasonography for pregnancy rate and collection of fawning data were conducted in fallow does in the same manners as described previously for axis does.

RESULTS

Transrectal ultrasonography on d 42 post-AI revealed that none of the axis does inseminated with fallow semen or fallow does inseminated with axis semen were pregnant as a result of the AI. Serum concentrations of P_4 on d 21 post-AI were 1.8±0.3 and 1.6±0.3 ng mL^{-1} for axis and fallow does respectively. Thereafter (d 28, 35 and 42) concentrations of P_4 were higher (Student's t-test; $p<0.05$) than at d 21, indicative of subsequent luteal function associated with diestrus and possibly the establishment of early pregnancies to natural matings following buck introduction (serum concentrations of P_4–axis does: 6.4±0.8, 3.9±0.9 2.4±0.3 ng mL^{-1}; fallow does: 2.6±0.3, 6.4±0.3, 4.6±0.4 ng mL^{-1} on d 28, 35 and 42, respectively). Serum concentrations of PSPB were undetectable at d 28 and 35 post-AI for all axis and fallow does, supporting serum P_4 and ultrasonography results indicating that no pregnancies had been established as a result of AI. Reproductive potential of the does was demonstrated when axis and fallow does were maintained with same-species axis and fallow clean-up sires for the 60-d breeding season in which pregnancy rates by ultrasonography were 93.8 and 89.5% for axis and fallow does, respectively, with subsequent fawning rates of 72.7 and 87.5%, respectively.

DISCUSSION

Artificial insemination is a practice that has been used in some deer farming systems to take advantage of superior genetics and to facilitate cross-breeding or hybridization for improved antler development and to advance offspring growth for improved venison production[9,16]. The laparoscopic AI techniques employed in the present investigation are commonplace and have been used previously by our laboratory for hybridization between wapiti and sika deer[16]. In the present investigation, this attempt at reciprocal hybridization between axis and fallow deer did not result in the establishment of pregnancies. This was confirmed by ultrasonography and serum concentrations of P_4 and PSPB. While serum concentrations of P_4 are not specific to pregnancy (i.e., luteal P_4 production is a hallmark of diestrus and pregnancy), concentrations of P_4 at d 21 post-AI were considerably lower for both axis and fallow does than values observed previously in our laboratory for pregnant fallow does around this same time (i.e., 5 to 6 ng mL^{-1} on d 23 post-mating;[17]. While subsequent concentrations of P_4 on d 28, 35 and 42 were higher than those observed at d 21, these concentrations are indicative of luteal activity during diestrus and possibly, later values (d 42) may be associated with extended luteal function as a result of transitions to early pregnancy following matings with natural (same-species) sires after buck introduction. While serum concentrations of P_4 are not specific to pregnancy, serum PSPB is a highly specific diagnostic indicator that is only present if a pregnancy has been established since it is of trophoblast origin[19,20]. Pregnancy-specific protein B has been previously reported to be a reliable indicator of pregnancy in red deer[21] and fallow deer[17] between d 27 to 33 following mating. Nevertheless, serum PSPB in this study was not detectable in axis or fallow does on d 28 and 35 post-AI, further indicating that hybridization and establishment of early pregnancies did not occur in this study. The reproductive potential of the axis and fallow doe herds used in this study were demonstrated post-AI following buck introduction in which pregnancy rates were 89.5 and 93.8% for fallow and axis does, respectively, indicating that doe fertility was not in question. While companion same-species AI was not conducted in this study as a control to this hybridization attempt by AI (due primarily to animal numbers), the semen quality used for AI and the success of previous AI attempts with hybridization of wapiti and sika deer[16] would suggest that this AI attempt to hybridize axis and fallow deer was given every opportunity to occur.

CONCULSION

Using the procedures for laparoscopic AI outlined in this study reciprocal hybridization of fallow and axis deer did not result in the establishment of hybrid pregnancies as confirmed by ultrasonography and serum concentrations of P_4 and PSPB. While anecdotal and undocumented accounts of the existence of such hybrids have been reported by some producers, none have been confirmed via genetic analysis or other empirical evidence of such crosses. Moreover, the absence of reports of hybridization among large mixed herds of fallow and axis deer in free-ranging environments where they have been introduced together would suggest that hybridization between these two species is highly unlikely; although the possibility of which cannot be completely discounted until further studies can be conducted (e.g. *in vitro* fertilization attempts) that may shed new light on the physiological mechanisms (genetic or otherwise) blocking the production of such hybrids.

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REFERENCES

1. Lowe, V.P.W. and A.S. Gardiner, 1975. Hybridization between red deer (*Cervus elaphus*) and sika deer (Cervus Nippon) with particular reference to stocks in N.W. England. Zool. Lond., 177: 553-566.
2. Bartos, L., J. Hyanek and J. Zirovnicky, 1981. Hybridization between red and sika deer: I. Craniological analysis. Zool. Anz. Jena., 207: 260.
3. Bartos, L. and J. Zirovnicky, 1981. Hybridization between red and sika deer II. Phenotype analysis. Zool. Anz. Jena., 207: 271
4. Bartos, L. and J. Zirovnicky, 1982. Hybridization between red and sika deer III. Interspecific behaviour. Zool. Anz. Jena., 208: 30.
5. Groves, C.P. and P. Grubb, 1987. Relationships of living *Cervidae*. In: Wemmer, C., (Ed.). Biology and Management of *Cervidae*. Smithsonian Inst. Press, Washington, DC, pp: 21-59.
6. Pearse, A.J., 1992. Farming Wapiti and Wapiti Hybrids in New Zealand. In: Brown, R.D., (Ed.). The Biology of Deer. Springer-Verlag, New York, NY, pp: 173-179.
7. Willard, S.T., D.M. Hughes, M. Brignans, R.G. Sasser, J.T. Jaques, R.W. Godfrey, T.H. Welsh, Jr. and R.D. Randel, 1996. Artificial breeding, hybridization and pregnancy detection in sika deer (*Cervus nippon*).Theriogenology, 46: 779-789.
8. Tate, M.L., R.M. Anderson, K.M. McEwan, G.J. Goosen and A.J. Pearse, 1998. Genetic analysis of farmed deer hybrids. Acta Vet. Hung., 46: 329-340.
9. Fennessy, P.F. and C.G. Mackintosh, 1992. Hybridization of red deer and pere David's deer. Proc. Deer Branch Course New Zealand Vet. Assoc., 9: 181-186.
10 Hsu, T.C. and K. Benirschke, 1967. An atlas of mammalian chromosomes–Dama dama (Fallow deer). 1: 41.
11. Hsu, T.C. and K. Benirschke, 1974. An atlas of mammalian chromosomes–*Axis axis* (Axis deer). 8: 390.
12. Whitehead, G.K., 1993. The Whitehead Encyclopedia of Deer. Swan Hill Press. Shrewsbury, England.
13. Mungall, E.C. and W. J. Sheffield, 1994. Appendix C. Hoofed Hybrids. In: Mungall, E.C. and W.J. Sheffield, (Eds.). Exotics on the Range: The Texas Example. Texas A and M University Press, College Station, TX, pp: 209.
14. Asher, G.W., D.S. Gallagher, M.L. Tate and C. Tedford. 1999. Hybridization between sika deer (*Cervus nippon*) and axis deer (*Axis axis*). J. Heredity, 90: 236-240.
15 .Mungall, E.C., 1994. Texotics tables census numbers. Exotic Wildlife Association Publication: The Fence Line 4: 14.
16. Willard, S.T., S. Chapman, G. Foxworth, M. Drew, D.M. Hughes, D.A. Neuendorff and R.D. Randel, 1998a. Hybridization between wapiti (*Cervus elaphus manitobensis*) and sika deer (*Cervus nippon*): A comparison of two artificial insemination techniques. J. Zool. Wildlife Med., 29: 295-299.
17. Willard, S.T., R.G. Sasser, J.C. Gillespie, J.T. Jaques, T.H. Welsh and R.D. Randel, 1994. A comparison of methods for pregnancy determination and the effects of body condition on pregnancy status in Rocky Mountain elk (*Cervus elaphus nelsoni*). Theriogenology, 42: 1095-1102.
18. Willard, S.T., R.G. Sasser, J.T. Jaques, D.A. Neuendorff and R.D. Randel, 1998b. Early pregnancy detection and the hormonal characterization of embryonic fetal mortality in farmed fallow deer (*Dama dama*). Theriogenology, 49: 861-869.
19. Reimers, T.J., R.G. Sasser and C.A. Ruder, 1985. Production of pregnancy specific protein by bovine binucleate trophoblastic cells. Biol. Reprod., 32: 65.
20. Sasser, R.G. and C.A. Ruder, 1987. Detection of early pregnancy in domestic ruminants. J. Reprod. Fertil., 34: 261-271.
21. Haigh, J.C., M. Cranfield and R.G. Sasser, 1988. Estrus synchronization and pregnancy diagnosis in red deer. J. Zool. Anim. Med., 19: 202-207.