Does excess circulating glucose in pregnant females drive male-biased birth sex ratios? – beginning a study of the world herd funding by the IRF

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Abstract: Male-biased birth sex ratios (BSR) are common in captivity and threaten meta-population viability. The literature on perissodactyl gestation and BSR was used to construct a predictive model of the effect of translocation on BSR perhaps via stress induced hyperglyceamia. I used rhinoceros international studbooks (white, *Ceratotherium simum*; black, *Diceros bicornis;* and Indian, *Rhinoceros unicornis*) to identify and categorise mothers translocated at different times during pregnancy and calculated their subsequent BSR. Mothers (n = 48) translocated during early gestation (conception to 0.2 gestation or approximately 66 days) had more male calves (71.4% male). Results indicate a switch from female embryo death in early gestation to greater male embryo vulnerability after placental dependence (>66 days) and support predictions that high circulating glucose in mothers and embryo glucose metabolism may be responsible for early female embryo death. Male embryo death from translocation during late gestation and subsequent female-biased BSR declined as the embryo matured. Results tentatively support the hypothesis that hyperglycaemia induced by stress and obesity cause extremely male-biased BSRs in captivity. This manuscript will motivate and inform experimental tests of our ability to manipulate BSR by managing stress and diet.

Keywords: facultative birth sex-ratio adjustment, rhinoceros, mammal, captive, glucose metabolism

1. INTRODUCTION

An aberrant male bias in the birth sex ratio (BSR) is a growing problem for the captive breeding programmes of many species. The problem has become particularly pronounced for some of the world's captive rhinoceros; e.g., white (*Ceratotherium simum*), black (*Diceros bicornis*), and Indian (*Rhinoceros unicornis*) rhinoceros [Atkinson 1997; Saville and Dennis 2005: Figure 1]. The BSR from endemic and minimally managed wild populations of black, white and Indian rhinoceros is 51.6% male but as high as 71% male in captive eastern black rhinoceros. Captive herds of the Javan (*Rhinoceros sundaicus*), and Sumatran (*Dicerorhinus sumatrensis*) species and the northern white (*C. s. cottoni*) and eastern black (*D. b. michaeli*) sub-species have a critical role to play in rhinoceros recovery and conservation because the future of their few small remaining wild populations is uncertain [Linklater 2003]. Rhinoceros males, like other polygynous mammals, contribute much less than females to reproduction

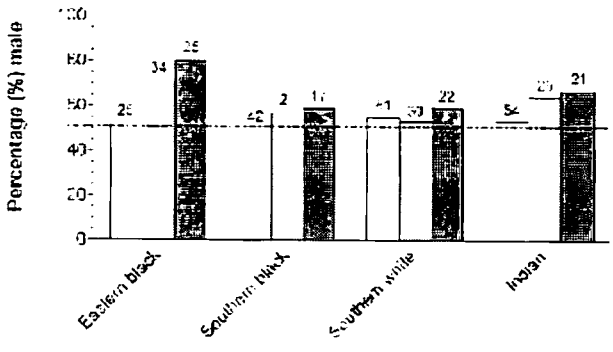

Figure 1. Wild (white) and captive (grey) birth sex ratio (% male calves), and surviving captive calf sex ratio (dark grey) for the eastern (*D. b. michaeli*) and southern (*D. b. minor* and *D. b. bicornis*) black, southern white (*C. s. simum*), and Indian rhinoceros [data compiled from Atkinson 1997]. The numbers above each bar are the number of calves that contributed.

and population growth, and their growing numbers in captivity utilise resources better targeted at

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females, particularly prospective mothers. Thus, a male bias in captive sex ratios reduces long-term reproductive rates and threatens the viability of regional meta-populations. The male-bias in captive BSRs could be reduced, and perhaps even managed towards an advantageous female-bias, if we knew the mechanism responsible for the different survival rates of male and female embryos and the factors that influence their survival most.

Recent work with bovine embryos suggests that excess circulating glucose in the mother and glucose metabolism by the embryo may be the mechanism that most influences BSR in mammals. Observations *in vitro* show male blastocyst development and implantation to be enhanced, but delayed for females, in glucose-rich media. Female blastocyst survival is poor, compared with that for males, in glucose-rich media [Larson *et al.* 2001]. The effect of glucose is attributed to several toxic metabolites that are by-products of the embryo's glucose metabolism [Kimura *et al.* 2005]. Glucose metabolism is controlled by genes on the X-chromosomes. In female blastocysts, the presence of 2 X-chromosomes ensures greater glucose metabolism, and thus higher concentrations of its toxic metabolites, before one of the X-chromosomes is eventually 'turned-off'. In male embryos, with only a single X-chromosome, the capacity for glucose metabolism and production of toxic metabolites is much less. Thus, female embryos might poison themselves if their intra-uterine environment is rich in glucose.

Two circumstances characteristic of captivity might influence concentrations of circulating glucose in a mother, and thus the glucose environment of her early embryo; stress and obesity. Stress and obesity can cause acute and chronic hyperglycaemia (i.e., excess circulating glucose) through the liberation of glucose from readily metabolised energy reserves and elevated nutrition. Managing captive rhino population for more female births may require reducing the incidence of stress and obesity in mothers during early gestation. Before making such a recommendation, however, we need to be certain that stress and obesity drive circulating glucose levels in rhinoceros mothers in the way expected and that glucose is the cause of male-biased BSRs. Being certain of these relationships will require experimental tests that modify the circulating glucose levels in pregnant rhinoceros during early gestation [Linklater *et al.* 2005]. However, the hypothesis and experimental design might be refined through an analysis of the rhinoceros studbooks.

The rhinoceros studbooks provide a fortuitous test of the potential influence of stress and obesity on BSR through sex-differential embryo death mediated by glucose metabolism. In this manuscript I confine the analysis to the relationship between stress and BSR. The capture of wild rhinoceros, their shipment, often internationally, and adjustment to captivity (i.e., wild to captive translocation) is a major stress event for pregnant rhinoceros and is known to cause, at least acute, hyperglycaemia [Kock *et al.* 1990a; Kock *et al.* 1990b]. However, while probable, the positive relationship between captivity, obesity and hyperglycaemia is not so well known for rhinoceros. I will return to the likely contribution of obesity in the discussion. The BSR of pregnant females that underwent translocation at different stages of gestation might be compared to test for changes in the relative rates of male and female embryo death. Enough is known about mammalian BSR variation and foetal development during gestation, particularly from the extensive work with horses (a Peissodactyla like rhino), to build a simple predictive model or hypothesis about the influence of stress events like translocation on BSR.

1.1. Building a predictive model

If hyperglycaemia causes greater early female embryo death then the translocation of pregnant mothers during early gestation should result in a male-biased BSR. The BSR from mothers challenged (e.g. poor nutrition) during late-gestation, however, can be female-biased due to greater mortality amongst male embryos [Kruuk *et al.* 1999] that are generally more vulnerable [Forsyth *et al.* 2004; McMillen 1979]. Thus, the impact of translocation (i.e., a major stress event) on the BSR should change depending on its timing relative to gestation. Translocation during early gestation should cause greater female, than male, embryo death whereas during late gestation a translocation should cause greater male embryo death. Therefore, translocation stress should translate into a male-biased BSR if occurs in early gestation, but a female-biased BSR if it occurs in late-gestation.

Exactly where in gestation the change from greater female to greater male embryo death occurs is not known. However, it might be predicted from what we know about embryo development. The cells of the small differentiating conceptus, blastomere and blastocyst are in direct contact with the mother's intra-uterine fluids, draw nourishment (e.g., glucose) from them, and rely on them to disperse toxic metabolites. As the embryo grows its cells not only become contained within a larger body with developing integument, but it develops a circulatory system for cell nourishment and metabolite disposal that is increasingly facilitated by the developing

placenta. Eventually the embryo is entirely dependent on the placenta for nourishment and waste removal.

In horses the placenta has fully assumed its role at about 100 days into their 343 day gestation [Allen 2001]. Thus, the switch in BSR from male- to female-bias in response to stress during early and late-gestation, respectively, should occur before 100/343 = 0.3 of gestation. I assume here that foetal development in rhinoceros species with average gestation times from 460 to 480 days can be scaled up from that observed in the horse with a 343 day gestation. Thus, our starting point in constructing the predictive model is to mark the time in gestation when we expect the change between the sexes in embryo vulnerability to stress to occur (i.e., the vertical line indicated by 'a' in Figure 2). In

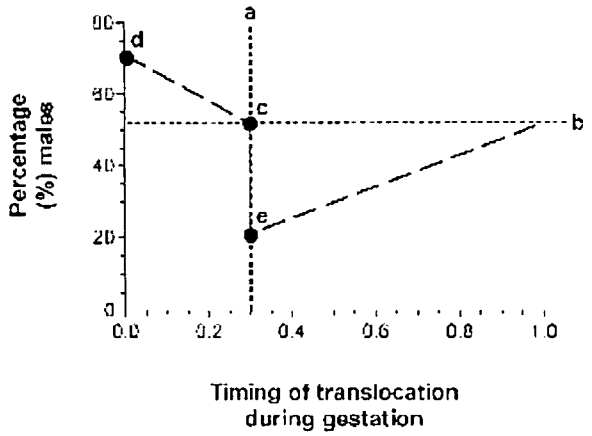

Figure 2. The predictive model, or hypothesis, describing the effect of translocation stress at different times during gestation on the subsequent birth sex ratio. See text for explanation.

constructing our predictive model, two other types of information are required that describe the shape of the model on either side of the 0.3 gestation timeline: (i) the slopes of the lines, and (ii) the magnitude of the effect: from conception at 0.0 to 0.3 gestation and 0.3 gestation to birth at 1.0 gestation. I have used the following figures and reasoning to construct the remainder of the predictive model shown (Figure 2) in the following way.

(i) Firstly, the slope of the line from 0.0 to 0.3 gestation should be negative because as the embryo grows, forms a relationship with the developing placenta, and becomes increasingly independent of the mothers circulatory and intra-uterine fluids, its vulnerability to high glucose concentrations should decline. If we assume that from the origin at 0.0 gestation the vulnerability of female embryos will converge with that of males towards parity (approximately line 'b', Figure 2) until the switch to male embryo vulnerability at 0.3 gestation (as indicated by point 'c', Figure 2), then the slope of the early gestation line is determined by the magnitude of the effect at 0.0 gestation (see ii below). Secondly, the slope of the line through 0.3-1.0 gestation must be positive since embryo sex ratio must converge with average BSR (i.e., approx. 51.6% male at 1.0 gestation; and the horizontal line indicated in Figure 2 by 'b') but describe poorer male foetal survival prior to birth. Thus, the magnitude of the female-bias (see ii below) describes the exact slope of the late gestation (0.3-1.0) line.

(ii) The potential magnitude of foetal death at different stages of gestation might be estimated from the most extreme female and male-biased BSRs observed in rhinoceros and wider perissodactyl literature. The largest statistically significant rhinoceros male-biased BSR is recorded for the captive eastern black rhinoceros population (Figure 1) at 71% male and is used as the origin of the model at conception (i.e., 0.0 gestation, indicated by point 'd', Figure 2). This figure and near parity at point 'c' describe the predicted slope of the model during early gestation (i.e., the line 'd – c', Figure 2). No statistically significant female biased birth sex ratios are known for rhinoceros. The largest statistically significant female BSR bias in a perissodactyl was recorded in New Zealand's Kaimanawa horses [Cameron *et al.* 1999] at 21% males born to mares with a below average body condition at conception. This figure is used to predict the greatest level of male embryo vulnerability after 0.3 gestation (indicated by point 'e', Figure 2) and along with the value at birth (indicated by line 'b') describes the predicted slope of the model during late gestation (i.e. the line 'e – b', Figure 2). The completed model is a highly simplified prediction. Many variants are possible should the susceptibility of embryos not be linear.

2. METHODS

We used the most recent international studbooks for black and white [1966 to 2004, Ochs 2005a; Ochs 2005b], and Indian [1965 to 2004, Hlavacek 2004] rhinoceros to identify all mothers (black n = 10, white n = 37 and Indian, n = 5) whose first calf born after arriving in captivity was born so soon as to have certainly been conceived in the wild and subject to translocation during its gestation. We recorded each calf's sex and backdated from its birth date to the mothers date of arrival in captivity to determine the time that the translocation occurred relative to gestation (using 460 days gestation for black rhinoceros and 480 days for white and Indian rhinoceros). The birth date of one white rhinoceros calf was labelled uncertain in the

studbook and therefore excluded from the analysis. Moreover, the sex of 4 calves, including the one of uncertain birth date, was unknown and did not contribute to the analysis.

The time taken to complete translocation from capture to captivity varied greatly between mothers and captivity is not necessarily the end of a female's translocation stress because she arrives to a novel social and physical environment. Nevertheless, arrival date in captivity was thought to be the most reliable and consistent index of the timing of the translocation event for all mothers. The time that each mother was translocated relative to her embryos gestation in days was converted into a fraction of the gestation length (i.e. conception at 0.0 to birth at 1.0). Each mother was placed into one of the following categories each spanning a fifth of the gestation period: 0.0-0.19, 0.2-0.39, 0.4-0.59, 0.6-0.79 and 0.8-1.0, depending on which period corresponded with her translocation. The sex of the mothers' calves in each gestation period was collated and the subsequent BSR (percentage male) calculated. The 4 unsexed calves that did not contribute to the BSR results were translocated when they were at 0.01, 0.52, 0.89, and 0.94 gestation and thus would have contributed to the first, middle and last gestation interval had their sex been known.

3. RESULTS

The BSR from mothers translocated at different times during pregnancy closely matched my predictive model of the effect of stress on rhinoceros BSR (Figure 3). The results suggest that stress causes greater female embryo death during early gestation but greater male embryo death during late-gestation. Mothers translocated during early gestation, prior to foetal placental dependence, had a strongly male biased BSR whereas mothers translocated after foetal placental dependence (late-gestation) had a female biased BSR. Thus, a switch from female to male embryo vulnerability does appear to occur before 0.3 gestation. Moreover, the magnitude and slopes of the effect in early- and late-gestation were similar to the predictions. Male embryos become less vulnerable to maternal stress as they aged after placental dependence. If the prediction and test data differ at all it is that the male bias from translocation during early gestation was larger than expected and the female bias from translocation during late gestation less than expected. Thus, female embryos were more vulnerable in early gestation and male embryos were less vulnerable during late gestation than expected from the largest male and female BSR biases recorded in the literature.

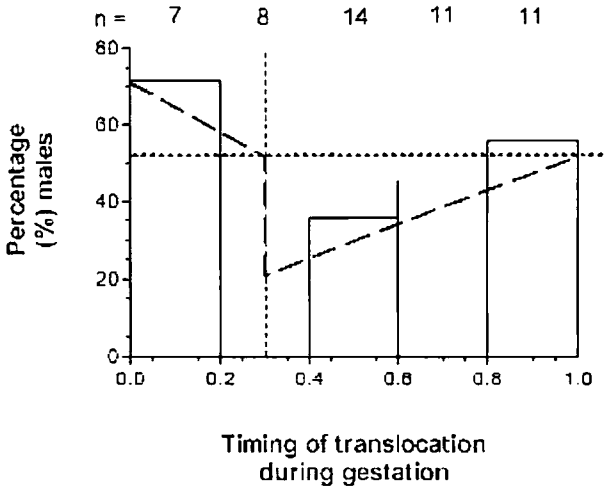

Figure 3. The BSR from mothers subject to the stress of capture and translocation from the wild into captivity at different stages of their gestation (bar). The number of calves that contributed to the BSR estimate is shown above each bar (n). The predictive model from Figure 2 (hatched and dashed lines) is overlaid with the data (bars) for comparison.

Although the results matched the predictive model, differences in the BSR due to translocation stress at different periods of an embryo's gestation were not statistically significant (G-test, $G = 4.0$, $df = 4$, $P > 0.25$), probably due to the relatively small number of rhinoceros females that qualified for the analysis (i.e. 48 sexed calves from mothers pregnant during translocation).

4. DISCUSSION

4.1. Stress, embryo death and BSR

Our results provide tentative support to the hypothesis that the relative vulnerability of different sexed embryos to stress switches from female to male, and changes in magnitude, as gestation proceeds. Importantly, we show that translocation stress at different stages of gestation translates into large differences in the subsequent BSR. In particular, the results support 3 key predictions:

(i) that in early gestation female embryos are more vulnerable to stress than male embryos and the magnitude of this effect can be extreme. An average 71.4% of calves (5 of 7) were male if the mother was translocated during the first 5th of gestation. The potential magnitude of stresses effect during early gestation on BSR must, however, be still larger than the 71.4% observed (or the largest male bias recorded in the literature; i.e., 71% male) because every embryo that survives the stress event must complete gestation during which time male embryos have greater death rates than females. Therefore, the male-bias in embryos generated from

a stress event during early gestation is likely to be reduced somewhat before birth. This has important implications for the managers of populations in captivity because the high-nutrition environment provided mothers probably reduces the difference between the sexes in their vulnerability during late-gestation. Therefore, the potential for male-biased extremes in BSR in captivity is greater than in wild or less resource-rich environments. Thus, our prediction and results are probably a conservative estimate of the male-biased BSR possible should pregnant females be subject to an extreme stress event during early gestation but be subsequently unstressed and super-resourced before parturition.

(ii) that in late gestation male embryos are more vulnerable to stress than female embryos and the magnitude of this effect is initially extreme but declines towards birth date. An average 25.0% of calves (2 of 8) were male if the mother was translocated during the second 5th of gestation. The observed rate was not as large as the predicted value and this is true for all periods of gestation. This supports the expectation above that the resource-rich environment of captivity reduces the differential between male and female embryo death during late gestation.

(iii) Lastly, our results provide first evidence that the switch from female to male embryo vulnerability to stress occurs before 0.3, and probably around 0.2 gestation.

The support provided for these predictions must be, however, regarded as tentative because of the small sample sizes used in this analysis and the lack of a statistically significant trend. Although the rhinoceros studbooks were large, the number of mothers that qualified for this study (i.e., were pregnant during their translocation) was too small to result in statistically significant differences between periods of gestation, test for the slope of the effect during early gestation, or more accurately identify the exact point that the switch from female to male vulnerability occurs. Nevertheless, this dataset might be increased by incorporating pregnant females that undergo significant translocations between distant institutions (i.e., captive to captive transfers). Moreover, we expect this pattern to be general for mammals, and thus this test could be replicated using the studbooks of very many other species.

4.2. The glucose mechanism

The likelihood that female embryos in early gestation are vulnerable to extreme stress events, like those experienced by pregnant females captured and translocated to captivity from the wild, lends support to the idea that excess circulating glucose might drive facultative BSR adjustment in mammals and be the cause of extremely male-biased birth sex ratios in captivity. The resource-rich captive environment probably elevates female body condition (sometimes resulting in obesity) and increases her average and/or peak circulating glucose levels with the potential for chronic hyperglycaemia. Moreover, females in captivity are periodically subject to significant stress events that are associated with population and meta-population management (e.g., the handling and/or immobilisation and confinement during husbandry and inter-exhibit or institutional transfer). A captive female's physiological response to stress inevitably results in acute hyperglycaemia that might be exacerbated by her elevated body condition and nutrient-rich diet. In captivity stress and obesity are likely to be synergistic, combining to produce extremely hyperglycaemic periods for females during early gestation. Thus, the circumstances are ideal for high rates of early female embryo death. When this effect is combined with the corresponding reduction in male embryo death (relative to that for female embryos) during late-gestation, then extremely male-biased birth sex ratios may result.

The results presented here from using the translocation of pregnant females during different stages of gestation as a fortuitous experiment in the effect of stress on BSR in rhinoceros, is a tentative test of the glucose mechanism proposed by Cameron [2004] to explain facultative BSR adjustment in mammals and why there is so much equivocal support for it in the literature. Our results confirm Cameron's expectation that BSR might be driven by events during early gestation that elevate the levels of circulating glucose in the mother.

4.3. Recommendations and future work

Managing rhinoceros BSRs is going to be critical to the future of captive populations and for their contribution to species recovery in the wild. These preliminary results indicate the importance of reducing stress for mothers in early gestation to limit the loss of female embryos. However, the wider hypothesis about the potential role of stress and obesity in male-biased BSRs via an embryos glucose metabolism still requires a definitive test, not just of its validity but also of our ability to manage a mother's glucose levels during the first 100 days gestation to increase the number of female calves born. This manuscript provides the theoretical framework to guide a 'field' test of this hypothesis with live rhino. The International Rhino Foundation has funded the author [and my collaborators: Nanz Zekala and Peter Law,

Linklater *et al.* 2005] to conduct just such a field test. The necessary experiment will require taking measurements of stress, body condition and glucose from many recently bred female rhino at several institutions. The diet and environment of some of those females will be managed for reduced circulating glucose while the remainder will be maintained at previous (if not elevated) levels. We invite institutions to contact us should they wish to be a part of this research.

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6. REFERENCES

Allen W.R., Fetomaternal interactions and influences during equine pregnancy, *Reproduction* 121(4), 513-527, 2001.

Atkinson S., Possible determinants of skewed natal sex ratios in captive black and Indian rhinoceros in North America: International Rhinoceros Foundation, 15 pp, 1997.

Cameron E.Z., Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism, *Proceedings of the Royal Society London B* 271, 1723-1728, 2004.

Cameron E.Z., W.L. Linklater, K.J. Stafford, and C.J. Veltman, Birth sex ratios relate to mare condition at conception in Kaimanawa horses, *Behavioral Ecology* 10(5), 472-475, 1999.

Forsyth D.M., K.G. Tustin, J.M. Gaillard, and A. Loison, Fetal sex ratio variation in the highly polygynous Himalayan tahr: evidence for differential male mortality, *Behavioral Ecology* 15(4), 572-578, 2004.

Hlavacek G., International Studbook 2004: Greater one-horned or Indian Rhinoceros *Rhinoceros unicornis* Linne, 1758, Basel, Switzerland: Zoo Basel, 72 pp, 2004.

Kimura K., L.D. Spate, M.P. Green, and R.M. Roberts, Effects of D-glucose concentration, D-fructose, and inhibitors of enzymes of the pentose phosphate pathway on the development and sex ratio of bovine blastocysts, *Molecular Reproduction and Development* 72(2), 201-207, 2005.

Kock M., R. du Toit, N. Kock, D. Morton, C. Foggin, and B. Paul, Effects of capture and translocation on biological parameters in free-ranging black rhinoceroses (*Diceros bicornis*) in Zimbabwe, *Journal of Zoo and Wildlife Medicine* 21(4), 414-424, 1990a.

Kock M., R. du Toit, D. Morton, N. Kock, and B. Paul, Baseline biological data collected from chemically immobilized, free-ranging black rhinoceros (*Diceros bicornis*) in Zimbabwe, *Journal of Zoo & Wildlife Medicine* 21, 283-291, 1990b.

Kruuk L.E.B., T.H. Clutton-Brock, S.D. Albon, J.M. Pemberton, and F.E. Guinness, Population density affects sex ratio variation in red deer, *Nature* 399(6735), 459-461, 1999.

Larson M., K. Kimura, H. Kubisch, and R. Roberts, Sexual dimorphism among bovine embryos in their ability to make the transition to expanded blastocyst and the expression of the signalling molecule IFN-tau, *Proceedings of the National Academy of Sciences, USA* 98, 9677-9682, 2001.

Linklater W.L., Science and management in a conservation crisis: a case study with rhinoceros, *Conservation Biology* 17(4), 968-976, 2003.

Linklater W.L., N. Zekala, and P. Law, 2005. Understanding the mechanism and causes of male-biased birth sex ratios in captivity: capitalizing on preliminary findings from investigations of black rhinoceros translocations. School of Biological Sciences, Victoria University of Wellington, 23 pp.

McMillen M.M., Differential mortality by sex in fetal and neonatal deaths, *Science* 204, 89-91, 1979.

Ochs A., International studbook for the black rhinoceros, *Diceros bicornis* (Linne 1758), Berlin AG: Zoologischer Garten Berlin AG, Volume 10, 233 pp, 2005a.

Ochs A., International studbook for the white rhinoceros, *Ceratotherium simum* (Burchell 1817), Berlin AG: Zoologischer Garten Berlin AG, Volume 10, 409 pp, 2005b.

Saville W., and P. Dennis, Executive summary: Advanced veterinary epidemiology training program focussing on the health of captive black rhinoceros: Ohio State University, 9 pp, 2005.