A cyclic pattern was evident In estrogen values measured in the two Indian rhinos; at the end of a follicular episode, estrone sulfate levels dropped and then some 40 days later rose again, stayed up for 7-10 days and thereafter declined again. When estrogens declined, pregnanediol levels increased, indicating the production of a corpus luteum (which secretes progesterone). Some 14 days later the pregnanediol dropped again. These measurements tied in with behavioral indications of estrus In these animals.

ESTRUS CYCLE DETERMINATION FROM CONDITION OF REPRODUCTIVE TRACT

Dr. Robert Wagner, veterinarian at the Pittsburgh Zoo. collected data from an 8 year old female southern white rhino over a period of 20 months. Attempts were made to collect samples biweekly. Behavioural observations for stage of estrus cycle were correlated with:

- 1. Rectal examination for uterine and cervical tone
- 2. Vaginal cytology
- 3. Urine hormones:
 - (a) total estrogens (estradiol-17-B, estrone and estrone sulphate)
 - (b) progesterone

Rectal examination of uterus and cervix revealed much Information about the female's cycle and anatomy. Ovaries were not palpable. The reproductive tract tone showed a change from being soft, pliable and flaccid to becoming firmer for some weeks prior to behavioural estrus, and then rapidly became well defined and turgid for two to four days during behavioural estrus.

Vaginal cytology was reported by Spellmore and Booth in AAZPA Regional Proceedings in 1981 for a black rhino. Similar findings were seen in the cytology of the white rhino. During diestrus, round non-cornified epithelial cells with distinct nuclei were seen, along with small quantities of mucus and debris. Then for about two or three days during proestrus the epithelial cells cornified and became angular In shape with pyknotic and darker nuclei. A slight increase in mucus and debris was noted at this point. Also at this time the cells began karyolysis and lost their nuclei. A sudden change at estrus in the non-cornified to cornified cell ratio (NC/C) often occurred within 12 hours; commonly, greater than 70% of the cells became cornified with considerable debris noted. The epithelial cells of estrus were then irregular in shape with edges folded over, and contained no nuclei. The NC/C ratio would revert back within 12 hours to 50/SO or greater with cells resembling new diestrus cells. Rapidly changing cytology seen In Pittsburgh's white rhino closely agrees with reports from San Diego Wild Animal Park of estrus lasting 15 hours based on behavioural observations (1985 SSP Survey).

Hormone analysis of urine for total estrogens and progesterones was completed as frequently as possible but occasionally time gaps of up to 12 days since collection would occur. Analysis was done by radioimmunoassay (RIA). Hormone concentrations were corrected for dilution by standardizing against creatinine levels. Baseline estrogen levels ranged from 200 to 900 pg/ml with small mid-cycle peaks of less than 900 pg/ml ranging between November and July. Total estrogens showed the best correlation with observable heat. Estrogen peaks of greater than 1 200 pg/ml occurred within four days of noted heat. From August to October multiple estrogenic peaks (less than 1 300 pg/ml) were seen with little pattern or regularity. During this time. poorly defined heats or no cyclic behavioural activity was seen. Progesterone peaks (0.12S-0.250 ng/ml) followed extremely close to declining estrogen peaks from November to July, then levels became erratic and poorly correlated. These hormone fluctuations may explain the lack of obvious estrus behaviour in Pittsburgh's female rhino from late summer to early winter. There seems to be a seasonal anestrus occurring in this female during this time.

From December to July, Pittsburgh's female has strong (easily observed) heats and regular estrus cycles. With approaching heat the uterus and cervix increase tone, the vaginal cytology changes from non-cornified to cornified cells and urine total estrogen levels peak. Behavioural estrus lasts three to five days. Progesterones rise after estrogen peaks and tone and vaginal cytology go back to baseline levels. Cycle length varies from 38 to 58 days with most cycles being 40 to 42 days. As mid-summer approaches, cyclic behaviour and observable heats are much harder to determine. This agrees with the non-cyclic activity in tract tone and cytology. Future goals are to isolate a LH-like compound in the urine, sonographic evaluation of ovaries for staging the cycle and eventually artificial insemination.

FURTHER RESEARCH ON METHODS FOR OVULATION AND PREGNANCY DETECTION

Dr. Richard Kock of the London Zoological Society presented results of studies done in collaboration with Dr. J.K. Hodges also of the London Zoo, on detection of ovulation and pregnancy in rhinos. The following is a summary of the results.

- Comparison of urinary estrogen metaboiltes during pregnancy. Sequential hydrolysis of urine samples from midlate pregnancy in the Indian, black and white rhino showed:
 - (a) important species differences in the amounts and type of estrogen excreted;
 - (b) large amounts of estrogens were detected during pregnancy in the Indian species. The most abundant estrogen component was estrone sulfate;
 - (C) very low levels of estrogen were excreted in urine during pregnancy In the black and white rhinos. Of those measured estradiol glucuronide appeared to predominate;
 - (d) measurement of urinary estrogens may be useful for monitoring pregnancy in the Indian rhino but not at present in the other two species;
 - (e) more studies are needed in the black and white rhinos to examine the presence of other urinary estrogens and to determine whether there is a preferential route of fecal excretion.
- Measurement of urinary progesterone metabolites during pregnancy.

Urinary pregnanediol-3x-glucuronide was measured during mid-late pregnancy in the Indian, black and white rhinos. The results showed:

- (a) elevated levels of PdG in all three species;
- (b) levels In the Indian rhino were between 5-10 ug/mg creatinine whereas levels in the black and white rhinos were much lower at comparable stages of pregnancy (0.4-0.8 ug/mg Cr and 0.05-0.1 ug/mg Cr, respectively);
- (c) levels of PdG in all three species fell markedly (greater than ten-fold) within one week of termination of pregnancy (birth or abortion);