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URINE COLLECTION:
VALUE IN REPRODUCTIVE RESEARCH

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Zoos have steadily shifted from primarily places of exhibition to institutions more concerned with conservation and propagation. Dwindling numbers and the difficulty of attaining animals from the wild has made their breeding in zoos imperative. Various techniques have been employed from exhibit manipulation to population management. Reproductive physiology is being increasingly investigated.

Artificial reproduction is the goal in some instances because natural means have been unsuccessful. Some zoos are utilizing semen collection. Many of the zoos that are collecting semen are also freezing it in hopes that it can eventually be used for artificial insemination. Semen has been obtained from many animals, but the female physiology of the species has not been investigated. Information is sparse or non-existant. It is very difficult to judge behaviorly when a female is in heat or even if she is pregnant. The best determinant is evaluation of her reproductive hormones.

Urine collection is a noninvasive evaluation of these hormones. It causes the least alteration due to minimal stress and gives the closest evaluations obtainable. Three most commonly used methods of evaluating the collection are:

1. Radioimmunoassay (RIA)
   the taging of hormones with radioactive material that can then be counted
2. Bioassay
   measuring the physical response of cells or organisms to hormone stimulus
3. Antigen-antibody assay
   such as the hemagglutination test in which a precipitate is indicative

These tests can lead to determination of the female cycle.

Besides judgement of the normal cycling of the animal, or state of pregnancy, specific determination can be made such as heat period and time of ovulation. In a reproductive problem normal cycling is one consideration in the complete evaluation of fertility. Once normal fertility has been determined, but circumstances still prevent production, artificial means may be considered. Then timing of ovulation will be instrumental. In the condition of pregnancy the animal may need to be isolated and provided the proper environment for parturition and rearing.

Some variety exists in the normal cycling of species especially considering season of heat periods, duration of estrus, and length of cycle, but hormonal patterns and timing of the peaks of hormones are very similar (Fig. 8-15). A theoretical composite shows the sequence of elevations (Fig. 1). These elevations of hormones can be determined through serial samples of hormonal metabolites in the urine. Peaks of specific hormones indicate cycle events. High levels of the progesterone metabolite pregnanediol indicate pregnancy while elevated levels indicate midcycle. Estradiol peaks during the heat period and luteinizing hormone is indicative of ovaulation.

The urine collection can be made in the morning by the keepers when they make their initial check on the animal or by utilization of a volunteer. Most animals urinate when they first rise in the morning. This sample is the most useful since it is a concentrated voidance. Keeper awareness and observations on behavior to correlate with hormone evaluations is very important. Volunteers can make observations as well as identify the animals that urinated in a large group. Volunteers can also be utilized to transport the sample to facilities to be cooled as soon as possible.

Contaminations of the sample should be kept to a minimum. Dirt can be eliminated later with centrifuging of the sample and is not such a problem as long as the sample is immediately cooled.

Collection of 3-4 cc of urine can be made with a 5 cc syringe or pipette and frozen in a Wheaton vial or red top blood collection tube. Any amount of urine is valuable since pooled samples can be tested. Glass storage vials are preferred since hormones may stick to plastic. These vials are then clearly labeled with indelible ink pens with the date, time of day, animal identification, and collectors initials. Scotch tape keeps the writing from wearing off the vial. If there is a freezer on sight it is very useful for keepers or collectors to store samples until they can be retrieved.

A data sheet should be kept making note of all information printed on the vial so an additional note of collection can be kept. On this sheet should be space for observations on animals, making note of behavior, illness, or gross changes in urine.

Once the samples have been collected, the hormones contained in the urine can be evaluated in several differnt ways. For pregnancy evaluation, the Subhuman Primate Pregnancy test is a hemagglutin test in which a precipitate is a positive reaction. It is not a quantitative test and can show both false positives and false negatives depending on the age of the test or sample. The test measures

chorionic gonadotropin which is cross reactive with luteinizing hormone (LH). The test can give a false positive when there is an LH peak. This test can be obtained for free and only drops of urine are required to run the test. This allows the rest of the sample to be saved for additional tests.

Radioimmunoassay (RIA) is the most effective test. It is a quantitative as well as qualitative test so cycle variation can be determined. The steroid hormones are the easiest to test since they are similar throughout species. The protein hormones such as LH are more species specific and may require specificity in testing material which is not available in the species. Okapi, rhino, and gorilla (Fig. 8,9) are examples of tests that have been preformed. The okapi chart demonstrates regular cycling with arrow indications of behavioral estrus. The rhino chart demonstrates an 80 day period with the dark bars indicating behavioral estrus. The gorilla graphs demonstrate the LH and estrogen peaks associated with ovulation. LH in this case was obtained through a bioassay.

Few zoos are doing RIAs as a routine. San Deigo is well established and the Bronx Zoo now has a full time endocrinologist. Ed Ramsey, DVM of the Oklahoma Zoo is collecting urine on rhinos and Janis Ott, DVM of the Brookfield Zoo is collecting urines on various hoofstock. Both of the hormone evaluation projects are being handled by the San Deigo Zoo laboratory. Large human labs will run samples sometimes for free and can be very indicative in the primates. However their use with other species is more involved.

Urine collection and assay have proven to be a useful tool in determination of normal hormonal values. As it expands greater understanding of the reproductive physiology of exotic species will follow. Pregnancy can then be evaluated, and the infertile animals can be isolated and treated. In addition, the effects of exogenous factors on the reproductive cycle can be examined. Once the timing of the female cycle can be established and influences dealt with, then if artificial means are required they can be better employed.

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[2]Hodges, JK, et al. Estrogen and Luteinizing Hormone Secretion in Diverse Primate Species from simplified urinary analysis. J Med Primatol 8:349-364 (1979).

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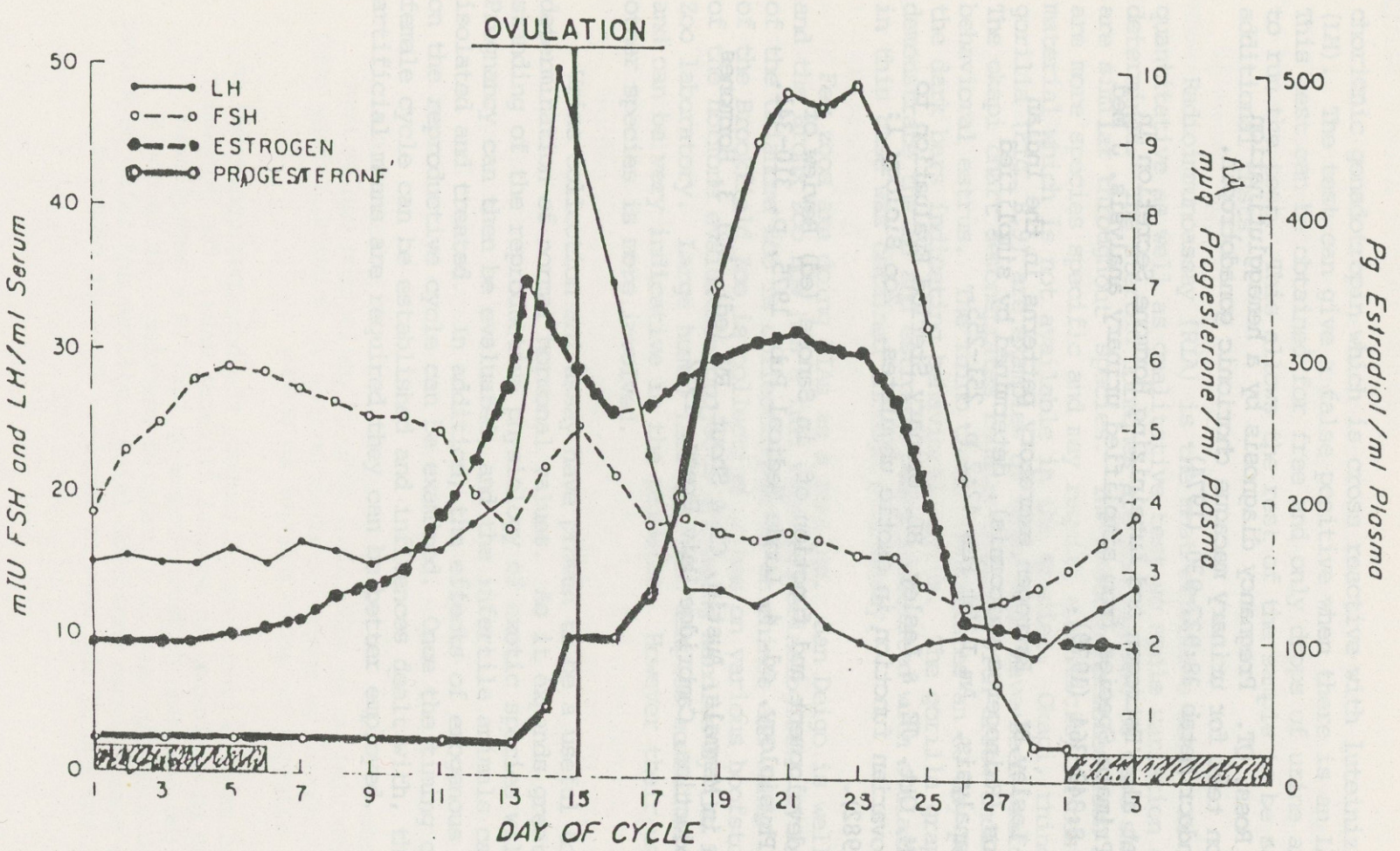

Fig. 1. Theoretical composite normal cycle demonstrating shifting values of FSH, LH, estradiol, and progesterone.

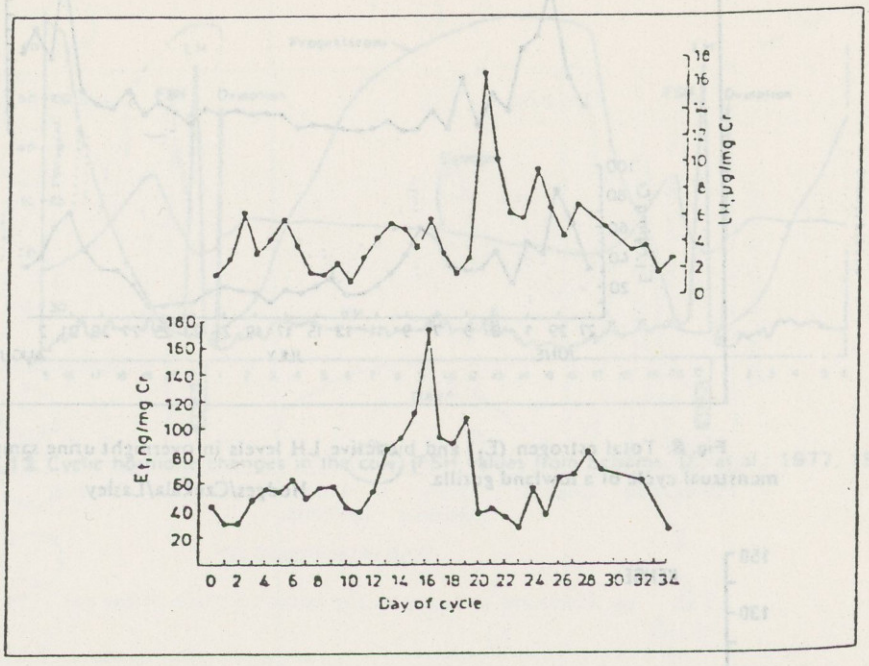

Fig. 2. Total estrogen (E_t) and bioactive LH levels in single morning voiding urine samples during a menstrual cycle of a chimpanzee. Hodges/Czekala/Lasley

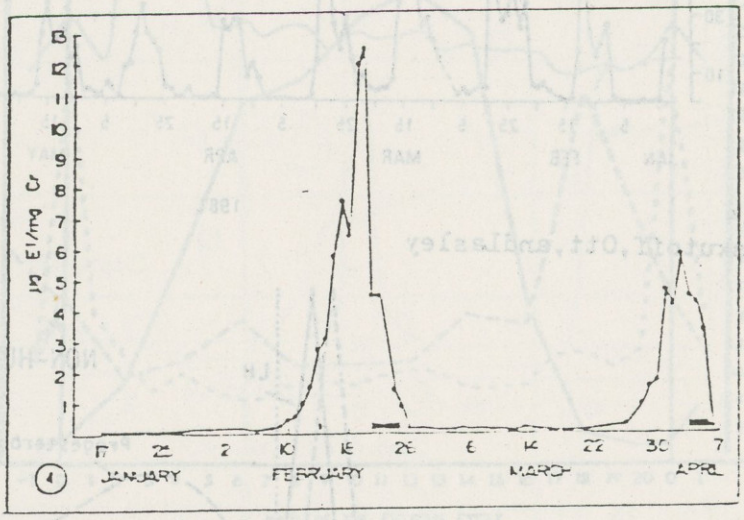

Fig 3 Nassar, and Lasley

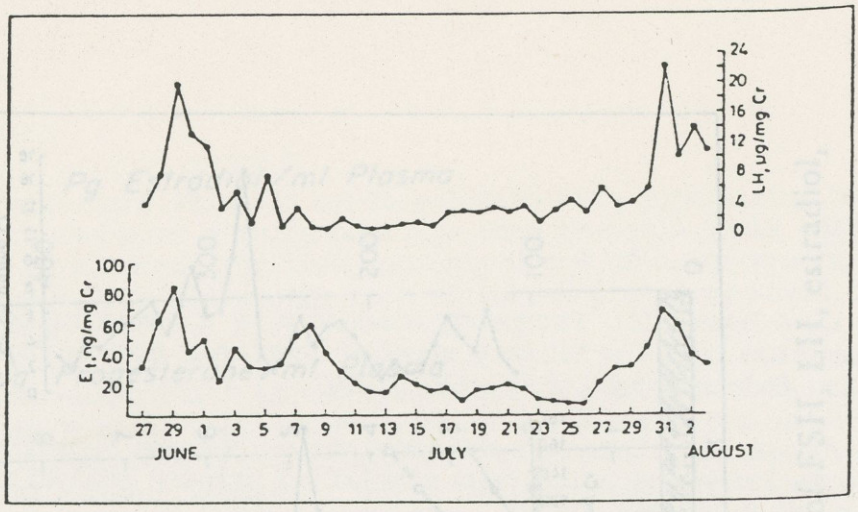

Fig. 8. Total estrogen (E_t) and bioactive LH levels in overnight urine samples during a menstrual cycle of a lowland gorilla.

Hodges/Czekala/Lasley

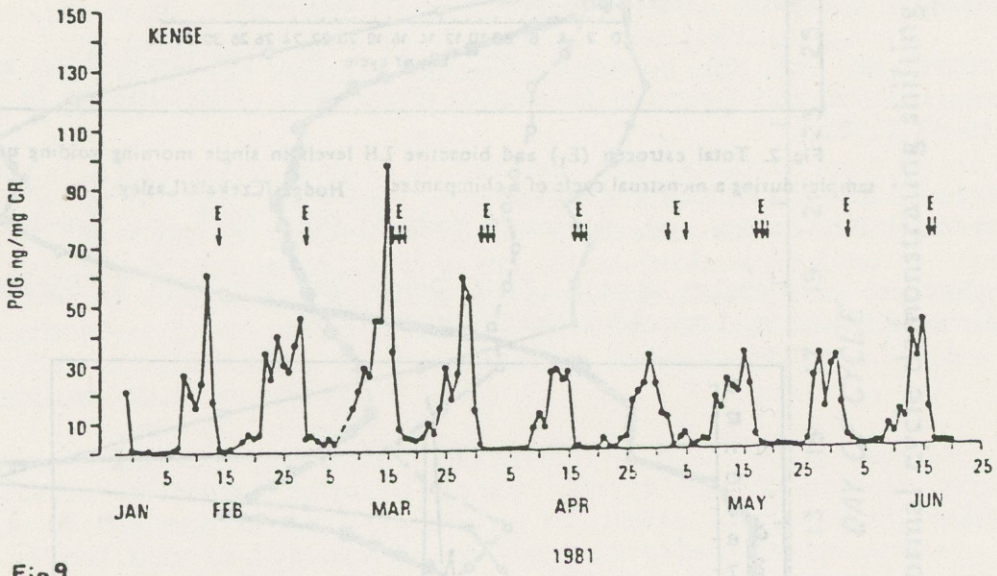

Fig 9
Loskutoff, Ott, and Lasley

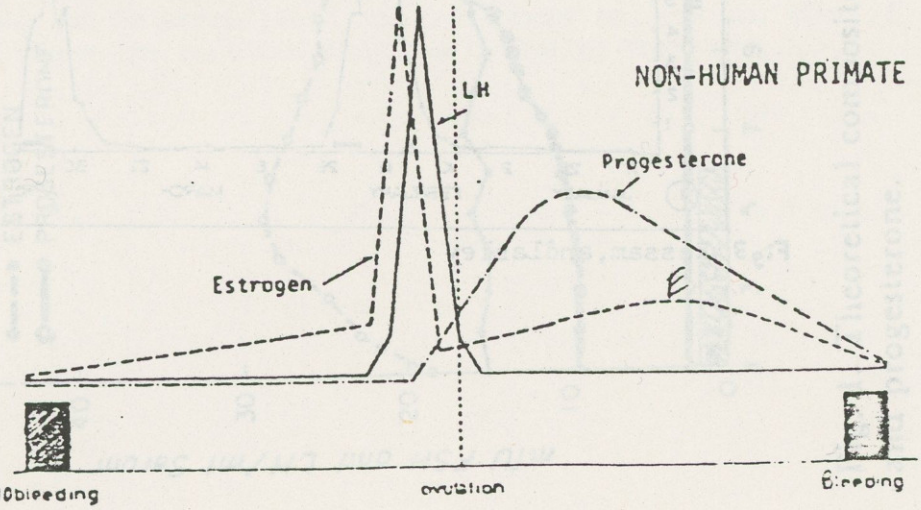

Fig 10 bleeding

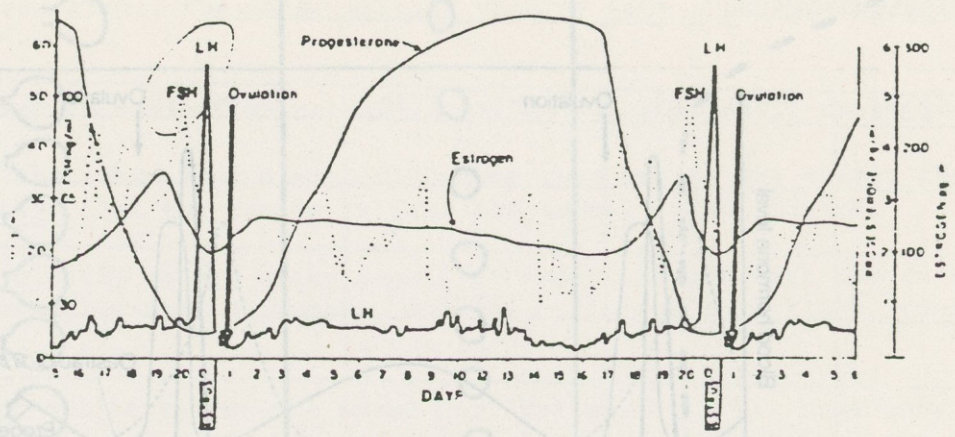

Fig. 12 Cyclic hormone changes in the cow (FSH values from Schams, D., et al.: 1977, 1978).

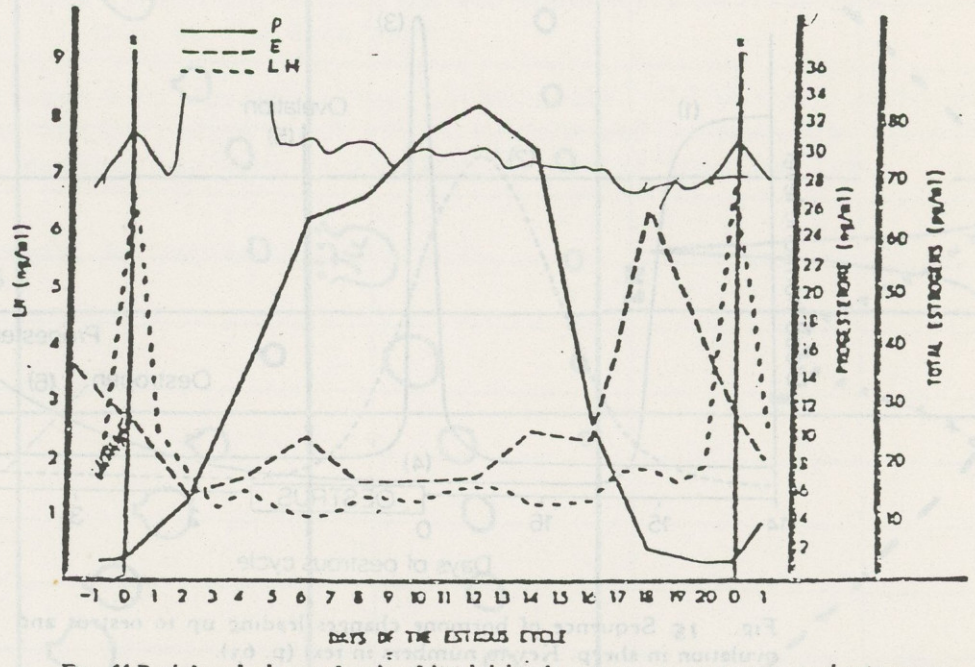

FIG. 11 Peripheral plasma levels of luteinizing hormone, progesterone, and estrogens during the porcine estrous cycle (Hansel *et al.*, 24).

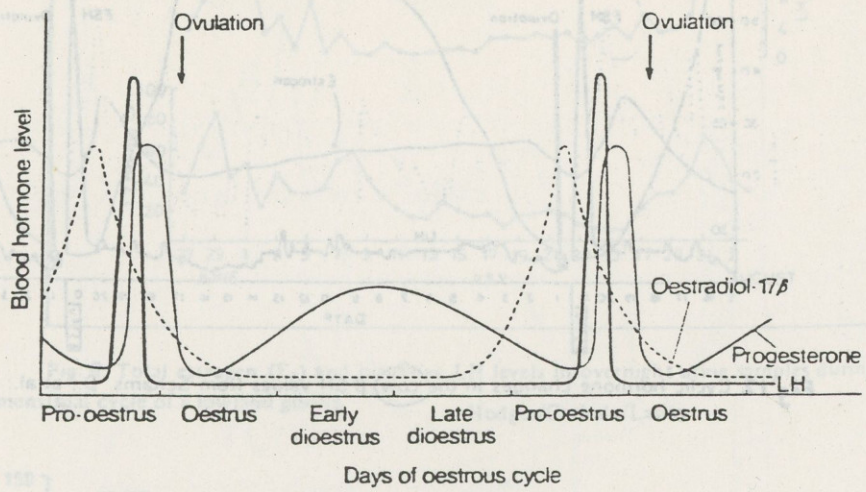

Fig. 14. Hormone changes in the oestrous cycle of the rat.

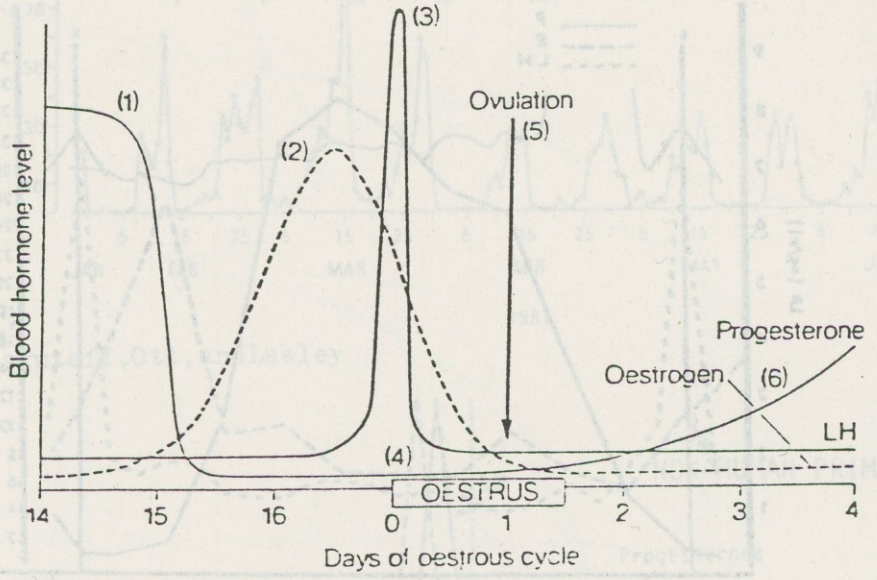

Fig. 15. Sequence of hormone changes leading up to oestrus and ovulation in sheep. Key to numbers in text (p. 63).

PALPABLE CHANGES DURING THE ESTRUS CYCLE

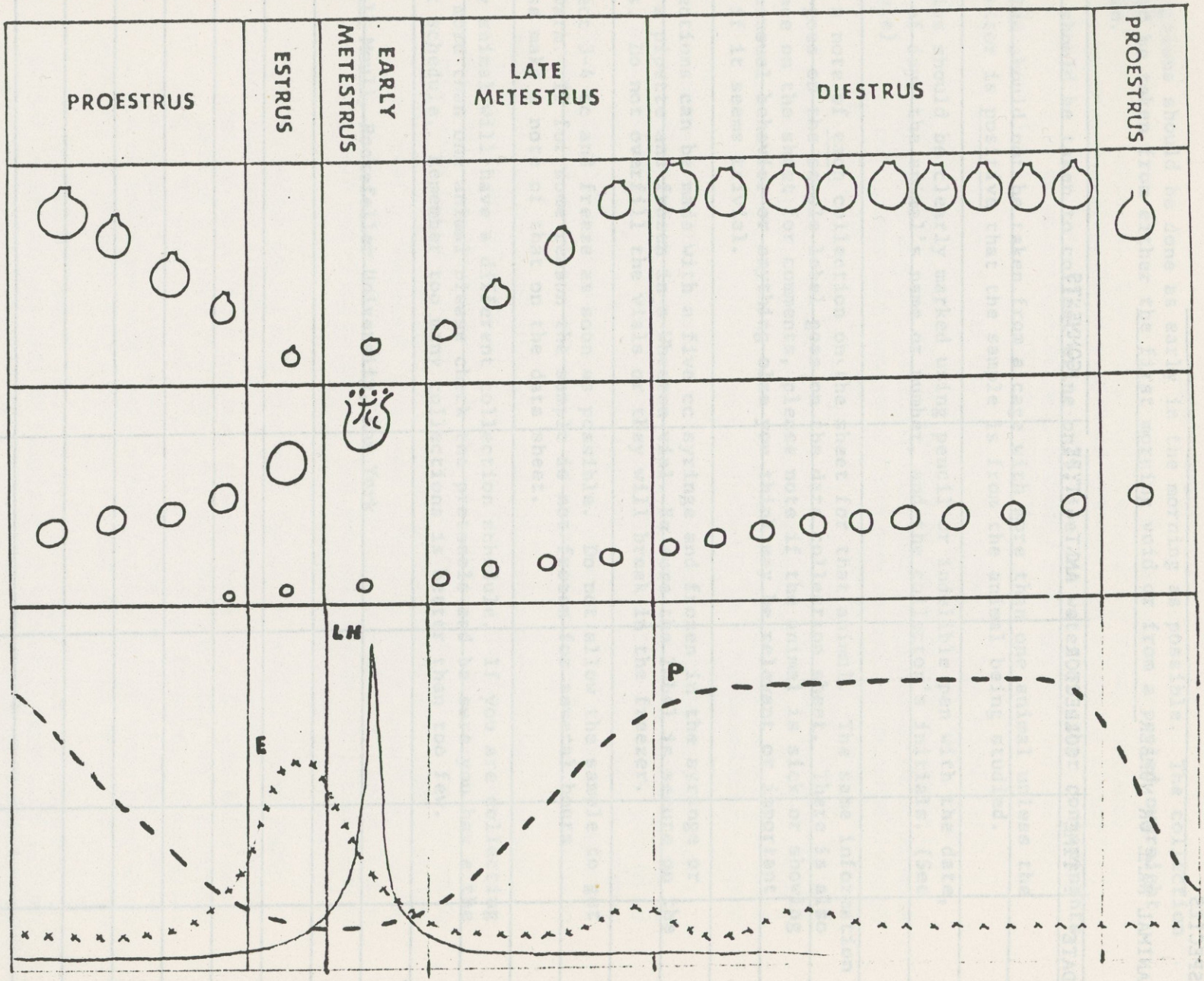

Fig 16

DATA COLLECTION SHEET

SPECIES _____

ANIMAL NAME OR NUMBER _____

DATE	TIME	COLLECTOR	AMNT & TYPE	COMMENTS

GENERAL PROTOCOL FOR URINE COLLECTION

Collections should be done as early in the morning as possible. The collection should be taken from either the first morning void or from a pooled overnight sample.

Care should be taken to collect urine only, i.e. no water or other contaminants.

Samples should not be taken from a cage with more than one animal unless the collector is positive that the sample is from the animal being studied.

Samples should be clearly marked using pencil or indelible pen with the date, time of day, the animal's name or number, and the collector's initials. (See example)

Make a note of each collection on the sheet for that animal. The same information that goes on the sample label goes on the data collection sheet. There is also a space on the sheet for comments, please note if the animal is sick or showing any unusual behavior or anything else you think may be relevant or important even if it seems trivial.

Collections can be made with a five cc syringe and frozen in the syringe or with a pipette and frozen in a Wheaton vial. Be sure the label is secure on the vials. Do not overfill the vials or they will break in the freezer.

Collect 3-4 cc and freeze as soon as possible. Do not allow the sample to get too warm. If for some reason the sample is not frozen for several hours please make a note of that on the data sheet.

Every animal will have a different collection schedule. If you are collecting from more than one animal please check the protocols and be sure you hav e the right schedule. Remember too many collections is better than too few.

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