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REVIEW

Faecal steroid metabolites assay as a non-invasive monitoring of reproductive status in animals

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Abstract

Measurement of faecal steroids as a non-invasive technique is widely used to monitor reproductive hormones in captive and free-ranging wild animals. This method offers a great advantage over invasive techniques like blood collection and deserves to be used in domestic animals. Repeated blood sampling is stressful, having animal welfare issues, difficult to obtain in field condition. In the faeces of cow, estradiol 17-α predominated, whereas in mares and sows, estradiol 17-β and estrone are main estrogens. Steroids are metabolized in liver, excreted in faeces and extraction is done by using a variety of methods such as ethanol, methanol etc. Faecal estrogen and progesterone metabolites evaluations are wel1 established approaches for monitoring of reproductive functions in a variety of mammalian species. So in future this method may be the most suitable for monitoring of reproductive status in farm animals particularly under field conditions.

Key words: Faecal steroids, non-invasive technique, estrogen, progesterone metabolites, reproductive status

Introduction

Determination of reproductive status is one of the most important factors for effective management in modern dairy industry. Captive management of any species benefits from understanding its basic reproductive biology, including endocrinology [1]. The non-invasive monitoring of hormones permit frequent sample collection and the alternatives are urine, milk and faeces. The estimation of urinary steroids has been used extensively to monitor the physiological status of animals [2,3]. The urine collection from farm animal species requires animal handling and fixing of catheters and milk can only be obtained from lactating animals, limits there uses for investigation particularly in heifers and dry-off animals. The faecal steroid metabolites opened up new windows for non-invasive monitoring of reproductive status. Therefore, faecal steroids are the most practicable choice for this purpose [4]. Studies of faecal steroid hormones were first conducted mainly on their metabolism and excretion [5], further studies done regarding the puberty, estrous cycle, pregnancy, abortion, reproductive behaviour, seasonality and the monitoring of treatment therapies in zoo and domestic animals [6].

Invasive Vs Non invasive methods

Invasive and non invasive monitoring of ovarian activity and pregnancy diagnosis have been done in domestic animals by determination of reproductive steroids in plasma/serum [7] and milk [8], urine [2], saliva [9] and faeces [10], respectively.

Excretion of faecal steroids

In general faecal estrogens are measured using specific estrogen antibodies or antibodies against total unconjugated estrogens. Most species voided faeces containing a higher percentage of free than conjugated steroids. Estrogens are end products of steroid metabolism and therefore the compounds in plasma and faeces are similar [11]. Progesterone is mainly metabolized by liver prior to its faecal excretion in ruminants [6]. About 99% of excreted progesterone is unconjugated compounds [12] and the principal faecal progesterone metabolites are 5α and 5β pregnane series in cows. The steroid hormone metabolites quantified in faeces, widely used in studies of wild animals, as a non invasive, non stressor, economical and animal saving technique which allows longitudinal studies by permitting frequent sampling of the same individual [13]. Such characteristics justify the use of this technique with laboratory and domestic animals. The route of excretion varies considerably among the species as well as between steroids within the same species. For example, in sheep 77% of progesterone metabolites excreted via faeces (12) in contrast 97% in domestic cat [13]. Several studies comparing plasma and faecal steroids value during the estrous cycle indicated a delay of faecal steroids excretion compared to that in plasma having a lag time of about 12-24 hour in ruminants and about 24 to over 48 hour in hind gut fermenters like horse, pig, rhino, elephant, primates [14]. It is 12 hour between milk progesterone and its faecal metabolites in cows. The delay time between the circulation of steroids in plasma and their appearance in urine samples rather short less than 5 hr, but faecal steroid metabolites have an appreciable lag time which approximately correlates with lag time necessary for the intestinal passage of bile to the rectum [12]. The lag time is affected by digestibility of the forage, which influences the rate of passage of digesta [15]. Rabiee *et*

al. [16] studied progesterone clearance with different diets and reported that changes in feed have less effect on faecal progesterone. Faecal progesterone metabolites after parturition did not decrease to basal value until 3 to 4 days [17] indicating the lag time. The difference in excretion time of steroid between the estrous cycle and postpartum is probably caused by the very high concentration present during pregnancy and by the enterohepatic circulation which retards the excretion [18]. Palme [19] addressed the main topic of concern in faecal steroid hormone metabolites such as sample collection and storage, time delay, extraction procedures, assay selection and validation as well as the confounding factors.

Extraction of faecal steroids

Extraction of the faecal steroids was done by Mostl *et al*. [11] and Hirata and Mori [20] in cattle by using diethyl ether while phosphate buffer solution was used by Hulten *et al*. [21] in gilts. Methanol was used in buffaloes [22], in cows [10,23] and ethanol by Capezzuto *et al*. [24] in goats. Recently extraction of fecal samples with petroleum ether has represented a valid alternative to other more time-consuming methods of determining faecal progesterone concentrations in the peripubertal female bottlenose dolphin (*Tursiops truncatus*) by Biancani et al. [25].

Faecal estrogen

Estradiol-l7 β is the primary and biologically active estrogen secreted both from theca and granulosa cells of growing ovarian follicles. Their effects on follicular growth are primarily mediated through estrogen receptor and expression of their receptor has been detected in granulosa cells of bovine antral follicles at various stages of its development [26]. Faecal estrogen determination was done in pregnant women [27], farm animals [11], bottlenose dolphin (*Tursiops truncatus*) [25], pichis Zaedyus pichiy (*Xenarthra: Dasypodidae*) [28], Gilbert's potoroo (*Potorous gilbertii*) [29], plains zebra mares [30], pygmy rabbits (*Brachylagus idahoensis*) [31], Snow Leopards (*Panthera uncia*) [32], Veiled Chameleons (*Chamaeleo calyptratus*) [33], chinchilla [34]. Faecal estrogen concentration was at peak on day 4 post estrus and the lowest during 2nd week of estrous cycle in buffalo [35] indicating a lag time of days 4 between serum and faecal concentrations, the same observation was reported by Kumar [36]. Although some studies reported the determination of the preovulatory estrogen peak in mares, but it was unsuccessful in cows which could be due to low plasma concentrations. It is a reliable indicator of ovulation in feline and canine species [37,38]. Faecal steroids has been used to monitor estrous cycle of giant panda (*Ailuro poda melanoleuca*) [39] and to evaluate ovarian function in Javan Gibbons (*Hylobates moloch*) [40] and Black-and-Gold Howlers (*Alouatta caraya*) [41]. Serum estradiol measures are poor indicators of follicular activity in elephants, whereas urinary estrogen analyses are capable of characterizing the two follicular waves that occur during the estrous cycle [42] and also possible that measurements of other steroid metabolites in serum, urine or faeces might reveal differences that were not evident by serum testosterone measures alone [43].

Estrone sulphate is the major estrogen produced by conceptus (feto-placental unit) and can be measured in maternal plasma, milk, faeces etc. in domesticated hoof stock, red buffalo, yak, Grevy's Zebra, Nubian ibex, musk ox, caribou, gorilla, orangutan, Mhorr gazelle, Przewalski's mare and Malaysian tapir, yellow baboon, sable antelope, bison, cynomologus monkey and wild felids [6,32,44,45] and could be a reliable indicator of fetal viability as well [11]. Pregnancy can be confirmed by both faecal and plasma estrogen after day 120 in cows and mares [46], after the 12th week in cows [47] and 14th weeks onwards in buffalo [36]. In sow, there is an estrogen peak in the blood between day 23-30 of gestation [48], which can be detected in faeces [49] as a method of pregnancy diagnosis. The applicability of non-invasive steroid hormones techniques for monitoring of reproductive status in wild boar with positive correlation to serum values, enabling a more informed and correct management of the species [50].

Faecal estrogen determination in the male can be applied to those species, which produce high amounts of estrogens in the testis, like stallion and boar. Faecal estrogen values of mature stallions can reach values compared to those of pregnant mares [46] and has been shown to be a reliable indicator of cryptorchidism in horses [51].

Faecal progesterone metabolites

Progesterone is principal steroid hormone secreted mainly by the corpus luteum (CL) that regulates estrous cycle and maintains pregnancy in all farm animals. Adrenal cortex and placenta also secrete some amount of progesterone during certain specific physiological stages like pregnancy. Faecal progesterone analysis has been successfully used for monitoring estrous cycle, pregnancy, abortion, puberty and seasonality in various animal species including primates [52], and wild animals mainly in caribous, rhinoceroses, felids, okapis, moose, minks, wolves, sable antelope etc. [6], brown brocket deer [53], Tsushima leopard cat [44] and recently in fishing cat (*Prionailurus viverrinus*) [54]. In African elephants, measures of progesterone and cortisol metabolites in faeces provide indices of reproductive function and physiological stress [55]. Monitoring of estrous cycle by faecal progesterone steroids in buffalo has been investigated by Arunji [35] who found pattern of faecal progesterone metabolites was virtually similar to that of plasma progesterone with delay time of days 2-4 and obtained faecal progesterone metabolites was basal on estrus and highest on day 8 of estrous cycle. Same correlation between serum and faecal progesterone was reported

by Kumar, [36] in buffalo. In buffalo, while studying the effect of Crestar™ on estrus synchronization, Hattab *et al.* [22] concluded that the progesterone concentration in blood clearly correlated with the concentration of metabolites in faeces, similar to finding of Kumar [36] and suggested that non-invasive method is a valuable tool for determining the luteal status.

Faecal steroid metabolites analysis has been successfully used for monitoring of reproductive status in mares [4], swine [21], cows [23], goats [24] and free ranging ungulates [56]. Rabiee *et al*. [57] studied plasma, milk and faecal progesterone concentration during estrous cycle of lactating dairy cows with different milk yields and concluded that milk production has no effect on faecal progesterone concentration. Isobe *et al*. [10] found that sensitivity and specificity of pregnancy diagnosis for beef and dairy heifers and beef cows was 71–79% at day 21–24 post-breeding by faecal progesterone metabolites which was comparable to the 72-77% in milk and serum progesterone profiles [7]. Kornmatitsuk *et al.* [23] measured faecal progesterone metabolites in dairy cows on day 19-22 post-breeding and found that it was 100% accurate in diagnosing non pregnant cows whereas accuracy of the pregnancy diagnosis was 67% and changes in faecal progesterone concentration were corresponding to serum values. Significance difference found between non-pregnant and pregnant buffalo on 3rd week was basis for as a tool for pregnancy diagnosis in buffalo by faecal progesterone assay [36] and Souza *et al.* [58] correlated faecal progesterone and estradiol metabolites with serum concentrations as well as physiological changes associated with pregnancy in *Cerdocyon thous.* Faecal progesterone metabolite analysis proved to be a reliable method for mapping oestrous cycle activity, but was not useful for the prediction of oestrus in captive southern hairy-nosed wombats (*Lasiorhinus latifrons*) [59]. Comparing vaginal cytology with faecal estrogen and progesterone metabolites, Frederick et al. [60] found that changes in genital appearance and behavior were consistent and complementary to hormonal data in sun bear (*Helarctos malayanus*). There was a significant positive correlation between plasma progesterone and dry faecal progesterone concentrations in goats [24], in buffalo [36] and in large hairy armadillo (*Chaetophractus villosus*) and crying armadillo (*Chaetophractus vellerosus*) [61]. The significant correlations between dry fecal and plasma progesterone concentrations validated this method for monitoring reproductive status in these species. Recently, Curry *et al*. [62] investigated the potential pregnancy biomarker proteins based on their increased abundance in the faeces of pregnant polar bears compared to pseudo-pregnant females (controls) promising a strong foundation for ensuing efforts to develop a non-invasive pregnancy assay for use in both captive and wild polar bears.

Conclusion

Determination of endocrine status by faecal steroids is one of the important tools for efficient management, and efforts to use assisted reproductive technologies like artificial insemination, synchronization and embryo transfer; diagnosis of reproductive disorders depend on the knowledge of basic reproductive physiology of a particular species. Faecal steroid analysis needs to be explored as a non invasive tool to monitor reproductive status of the animals keeping in view of field application.

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