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Mammary gland biopsy of Sahiwal-Friesian cows

(Biopsi kelenjar susu lembu betina Sahiwal-Friesian)

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Key words: biopsy, mammary glands, Sahiwal- Friesian

Abstrak

Kaedah biopsi memudahkan penilaian morfologi dan fungsi kelenjar susu daripada sejumlah lembu betina yang banyak. Pendekatan ini akan menjadi alternatif yang berdaya maju bagi mendapatkan tisu selepas sembelih. Teknik biopsi kelenjar susu lembu Sahiwal-Friesian dibentangkan dengan menggunakan kaedah sedasi (penenang) dan anestesia setempat. Teknik ini melibatkan pengambilan sebanyak 5 g sampel tisu kelenjar susu daripada tetek dengan menggunakan pisau skalpel. Lembu dibaringkan, kepalanya disokong bagi menghindar cecair rumen mengalir ke dalam saluran pernafasan. Langkah kawalan diambil dengan mengikat urat darah bagi memastikan haemostasis berlaku pada aras yang minimum. Antibiotik disuntik ke dalam otot selepas pembedahan. Pemulihan selepas pembedahan agak cepat tetapi tidak seragam. Tiada mastitis klinikal dicerapkan pada lembu yang dibedah di sepanjang tempoh laktasi. Pada lembu yang sama, pengurangan pengeluaran susu pada kelenjar yang di bedah dengan kelanjar kawalan tiada apa-apa perbezaan. Pengeluaran dan kandungan susu kembali ke aras seperti sebelum dibedah, manakala pengeluaran susu pada kelenjar yang tidak dibedah tidak terjejas. Biopsi pada satu kelenjar susu mengambil masa selama 20 minit dengan sedasi secukupnya.

Abstract

Mammary biopsy method facilitates assessment of mammary morphology and function from relatively large number of cows. In many mammary gland experiments, this approach would be a viable alternative in obtaining tissue post-slaughter. A technique for mammary biopsy in Sahiwal-Friesian cows is described using sedation and local anaesthesia. The technique involves approximately 5 g taking of tissue samples from the quarters of the udder with the use of the scalpel blade. The cow was casted on the ground with the head supported to prevent regurgitation of ruminal fluid into the respiratory tract. Preventive measures were taken to ensure minimal haemostasis with ligation. Adequate antibiotic cover (long acting antibiotic) was given intramuscularly following surgery. Post-operative recovery was uneventful and rapid. No clinical mastitis was observed in any of the biopsied cows throughout the remainder of the lactation. The loss of milk yield was no greater in biopsied glands than in control glands of the same cows. The milk yield and composition were rapidly

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and completely restored to pre-biopsy levels in the biopsied cows and from the normal quarters of the same cows were unaffected. Biopsy of a single quarter usually took about 20 minutes with adequate sedation.

Introduction

Mammary biopsy has proven to be a valuable tool in the study of mechanism controlling milk synthesis, mammary development and resistance to intramammary infection. Many attempts have been made to obtain fresh mammary tissue samples for histological and biochemical studies. However, the mammary gland being very vascular has made it difficult to remove sample tissue because of haemorrhaging and development of mastitis, which can adversely affect milk yield. Various researchers have reported a number of approaches for biopsy of the mammary tissue (Stelwagen and Grieve 1990; Knight and Peaker 1984; Platanow and Blobel 1962). To permit detail investigation of the mammary tissue, Knight and Peaker (1984) demonstrated that a 5 g biopsy sample of goat lactating tissue was representative of the secretory tissue mass as a whole. The biopsy technique also reduces the number of experimental animals required because there is no need to slaughter the animals.

A bovine biopsy technique was described by Hibbert (1964) using a sharpened cannula that could provide sufficient material (5–12 g) for investigation, but this method resulted in mastitis in two of 12 cows, and the recovery period was up to 18 days which is too long for most experiments. Andberg and Karlson (1942) also described a method for taking core samples of bovine mammary tissue. However, an 8 cm skin incision was necessary to retrieve the core once it had been cut and healing took up to 4 weeks. On the other hand, Knight and Peaker (1984) biopsied the goats' mammary glands under general anaesthetic and showed that full recovery within 72 h of the secretory activity, as measured by milk yield. General anaesthetic can be relatively easy to administrate and manage in smaller animals, hence a number of biopsies can be carried out in a day. Using general anaesthetic in large animals (cattle) is more difficult because of their physical size and the space necessary for recovery limits the rate at which animals can be biopsied. To perform fat biopsies in steers, Raptopoulos and Weaver (1984) used intravenous xylazine which was sufficient to provide the necessary analgesia required. Byatt and Bremel (1986) working on pregnant heifers reported that they obtained good biopsy samples but gave no indication whether their method would be suitable for lactating animals. The objective of the present paper was to describe a biopsy procedure that would remove a representative sample of the alveolar tissue through a small incision on lactating Sahiwal Friesian cows under field conditions, using xylazine together with local anaesthetic that allows rapid recovery of secretory function.

Materials and methods

Biopsies were performed on 50% (blood level) lactating Sahiwal-Friesian crossbred cows. The average lactation number (n=13) was 4.2 (s.d =1.69; range 2 to 7). Prior to the sampling, mammary glands were examined for any physical defects and mastitis. The cows were managed under free grazing system and concentrate (3 kg/head) was given during milking. The cows were milked in a conventional herringbone parlor, twice a day at 6 a.m. and again at 3 p.m.. Milk yield was recorded at each milking to an accuracy of 100 g. Ten similar cows in the same herd were also monitored for milk yield and composition over the sampling period as a control.

Surgery

Cows were starved for about 20 h prior to surgery and machined milked within 4 h before surgery. The same cows were further milked with two spaced intravenous injections of 5 units of synthetic oxytocin (Syntocin, 10 iu/mL oxytocin) given to ensure complete emptying of the udder. The animal was brought to the treatment crush for inducing anesthesia. Surgery was performed in an open space available near the cowshed, under sedation using xylazine (-5,6 dihydro-4H-1,3-thiazine–hydrochloride, Rompun solution 2%, Bayar Leverkusen, Germany), injected intramuscularly at 0.25 mg/50 kg body weight. Atropine (Atrosite, 0.65 mg/mL atropine sulphate, Troy laboratories PTY. Ltd. Australia) was given to inhibit salivation. The animal was casted on the right side within 10 minutes after the injection. The feet were restrained for safety and to allow clear access to the udder. The head was raised and supported to prevent any discharge from the rumen going into the respiratory tract. Under the normal surgical principles and sterilization technique, the left side of the udder and under side of the left hind leg was shaved and washed (about 10 cm^2 area). This area was then cleaned and made aseptic using tincture of iodine. Biopsy site was selected in the basal (upper) portion of the udder by palpation, avoiding fat and larger subcutaneous blood vessels wherever possible. The area was anesthetized by a subcutaneous injection of xylocaine 2% (Jurox PTY Ltd. Australia). An L-block anesthesia was performed to provide regional analgesia to the site of incision. Two biopsies were obtained simultaneously, one from each of the left fore and hind quarters. Deep sedation lasted for 30–40 minutes followed by a period of light sedation.

To gain entrance to the alveolar tissue, a 2–4 cm incision was made through the skin, deep enough to expose the pink-yellow parenchyma. Entry into the capsule (about 1 cm thick) by blunt dissection was achieved and the mammary secretory tissue was finally exposed. Then, 5–10 g portion of the secretory tissue was grasped with a hemostat, excised with a scalpel. Haemostasis was achieved with artery forceps and ligation using 3 metric catgut, aided by temporary packing of dry swaps. Extreme precaution was taken to ensure a complete haemostasis as possible, because this is a pre-requisite for rapid recovery of mammary function. The incision was closed by simple suture for the deep tissues (4 metric catgut), while interrupted blanket sutures using 6 metric nylon was used to close the skin incision. All animals were intramuscularly treated immediately with long acting antibiotics, oxytetracylcine (Terramycin/LA–25 mL (1 mL = 200 mg Terramycin/LA, Pfizer Inc. New York, USA). Before the actual biopsy study, three non productive animals were used for familiarizing the techniques. The findings from these cows are quote as 'unreported'.

Post surgical care

The surgery usually took about 40–50 minutes to complete for both the quarters. Soon after the surgery, the animals were brought to a standing position. Although the animals could stand up, their gait were unsteady because they were still in the light sedation state. All the animals, with the exception of the first cow, recovered rapidly after surgery and returned to normal milk production within 48 h. The first cow was still in deep sedation phase and took about 15 minutes to stand up. When the cow was walked to its shed, it again laid there for another 60 minutes. The surgical sites were observed for myasis and an anti-fly repellant was sprayed daily until the wound healed. The appetite of the animals returned within 2 h. This was evident when they started to chew the grass offered to them.

Milk composition

Milk samples from the morning milkings were taken daily for the estimation of milk fat, protein and lactose concentration.

Measurements of the milk composition was performed on the Milk-O-Scan milk analyser.

Statistical methods

Biopsy treatment effects related to milk yield, fat, protein, and lactose were evaluated by comparison of means for pretreatment with post treatment data using paired *t* tests. For comparison of weekly milk yield, fat, protein, and lactose between the normal and biopsied cows, ANOVA was used.

Results and discussion

Previous studies of mammary development have entailed killings groups of animals or even single individuals at different stages of pregnancy and lactation for various measurements and calculations (Jones 1979; Anderson et al. 1981). Further, one needs to use a large number of expensive animals in order to reduce effects of inter-animal variation and to increase reliability of the results. The major disadvantage of this type of approach is the cost. The present study indicated that biopsies could be successfully carried out under field condition without the use of sophisticated surgical rooms, hence we were able to produce conclusive results using relatively few animals which did not need to be killed. Provided aseptic conditions are observed, it is possible to prevent any post surgical complications. All the biopsies were carried out close to the cow shed. Sufficient quantity (about 5 gm) and quality of mammary tissue was obtained consistently with the present method. There was always a variable amount of fat and connective tissue with the samples but these were trimmed off to leave behind a good amount of secretory tissue. Secretory tissue was obtained from the 11 of the 12 quarters which were biopsied.

Intramammary bleeding was the major problem with the biopsies. Attention was given to the removal of blood clots by hand at subsequent milkings so that there was no carry-over effects on the milk yield.

Figure 1 shows milk yields for the biopsied and non-biopsied quarters of the 12 cows undergoing the full surgical procedure and also includes control animals on the same management routine without any fasting or surgery. Milk yield gradually increased after the biopsy and the recovery of the milk yield was similar for biopsied and non-biopsied quarters. This recovery was observed within 48 h. There was a short period of milk depression just after biopsy and this could be due to the effects of starving prior to surgery.

In a separate (unreported) trial of three biopsies, betamethasone (Betsolan, Pitman-Moore Ltd., Uxbridge, UK) was given just after biopsy to reduce tissue oedema. The steroid was given for 3 days but we found that the milk yield was reduced by 40% for 4 days. The animals were able to recover their milk only after 10 days *(Figure 2)*. There was no observable difference in the tissue oedema after biopsy whether betamethasone was used or not.

Milk composition was measured for milk fat, protein and lactose concentration. Infra red milk analyser was used to measure these parameters. None of the milk samples showed significant differences in fat, protein and lactose concentration. The overall mean milk fat, protein and lactose concentrations

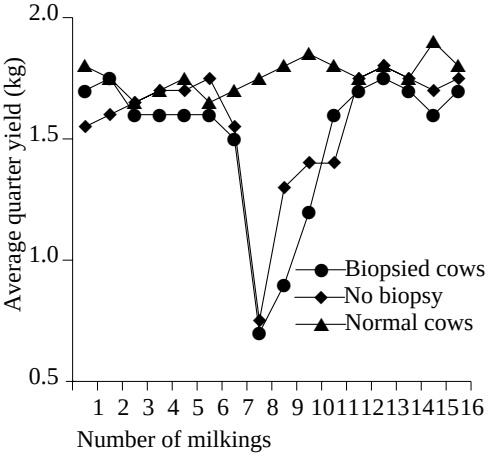

Figure 1. Average quarter yield at milkings every 12 h for normal, biopsied and non-biopsied quarters

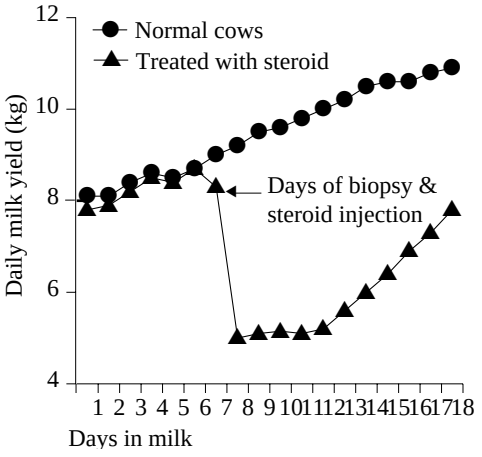

Figure 2. Milk yield of cows biopsied and treated with steroid (betamethasone).

are shown in *Table 1*. There was no significant (p >0.05) difference between the overall mean values for milk fat, protein and lactose concentrations of the normal cows when compared with the biopsied cows.

Initially milk from a few quarters contained some clots and frank blood for up to 24 h. However, careful hand-stripping removed clotted blood from the glands and visual examination of milk samples indicated that at 12 h intervals all the animals recovered fully. All the biopsy site wounds healed well and none of the biopsy sites became infected. This was due to the strict sterile method adopted in performing the surgery as well as adequate administration of antibiotics both locally and systemic. All the suture sites healed uneventful. There was also no incidence of mastitis in the biopsied animals. The secretory function of the biopsied quarters was seen to be maintained in all the animals. During the initial three biopsies (these were performed on a trial basis and is unreported) the biopsy site in one of the cows' became infected despite administration of antibiotics. Suture abscess was seen at the site but it healed rapidly. Subsequently this quarter resulted in clinical mastitis. The animal was then treated clinically for mastitis. Later it was found to have a significant loss of secretory function in this quarter. Similar incidences of abscess formation was reported by Oxender et al. (1971).

From the above study it is clear that mammary biopsy can be performed successfully in lactating cows under field conditions provided strict sterile procedures are followed. This procedure requires only deep sedation and local anesthesia and the recovery after biopsy can be rapid and with no adverse effects on secretory tissue.

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Table 1. Least sum means for milk fat, protein, lactose concentration of biopsied and normal crossbred Sahiwal-Friesian dairy cows

Treatment	LSM milk composition (g/kg)*		
	Fat	Protein	Lactose
Normal cows	35.1 ± 1.87	36.5 ± 1.29	41.3 ± 1.41
Biopsied cows	32.8 ± 1.92	37.9 ± 1.26	40.1 ± 1.36

*There was no significant difference for all the parameters measured (p <0.05).

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