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Original article

Uterine collagen during pregnancy in cattle

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Summary — Uteri were collected at the slaughter of non-pregnant dairy cattle and cattle at various stages of gestation. The weight of the whole uterus increased about 12-fold during the 9 month gestation period. The greatest increase was in the weight of the pregnant horn. The increase in weight of the uterine horns was accompanied by an increase in collagen content. The collagen concentration did not change. The collagen crosslink pyridinoline was identified in pregnant uterine tissues at a level of about 0.13 residues per mole of collagen; this level was the same at the beginning and end of pregnancy.

uterus / collagen / pregnancy / cattle

Resumé — Collagène utérin durant la gestation chez la vache. *Dans une enquête menée au niveau des abattoirs, ont été collectés des utérus appartenant à des vaches laitières non gestantes et gestantes à différents stades (du premier au 9e mois). Le poids de l'utérus a augmenté d'environ 12 fois durant la période des 9 mois de gestation ; l'augmentation la plus importante concernait la corne gravide. Cette augmentation du poids de la corne a été accompagnée d'un accroissement du contenu en collagène ; cependant la concentration du collagène n'a montré aucun changement. La liaison pyridinoline du collagène a été identifiée dans les tissus utérins de la vache gestante à un niveau d'environ 0,13 résidus/mole de collagène. Ce niveau était le même aussi bien au début qu'à la fin de la gestation.*

uterus / collagène / gestation / vache

* Correspondence and reprints

INTRODUCTION

During *post partum* involution of the uterus in cattle, there is a loss of connective tissue consistent with the overall reduction in tissue mass. This loss of collagen has been monitored from the urinary excretion of hydroxyproline and the collagen crosslink pyridinoline (Kaidi *et al*, 1990). That report indicated that increased *post partum* hydroxyproline excretion could be accounted for entirely by breakdown of uterine collagen. To add support to this suggestion it was necessary to confirm that there was an equivalent increase in the collagen content of the bovine uterus during pregnancy and to determine the nature of pyridinium crosslinks in uterine collagen during pregnancy. The results of such investigations are described in the present study.

MATERIALS AND METHODS

Bovine uteri

Thirty uteri were obtained from adult dairy cattle slaughtered at various stages up to 9 months of gestation as part of an abattoir survey of female reproductive tracts. The stage of pregnancy was estimated by measuring the foetal crown to rump lengths (Arthur *et al*,1989). In addition, 5 uteri at term were obtained from pregnant cattle killed as part of an experiment at the Agricultural and Food Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, UK.

In each case, the empty uterus (cervix, uterine body, left and right horns) was weighed, and then divided into its component parts. In animals more than 2 months pregnant, in which caruncles were sufficiently obvious, the caruncular and non-caruncular portions of each horn were weighed separately as well as the uterine body and the cervix.

For collagen analysis, 2 caruncles were removed from each part and replicate full thickness samples (2 x 2 cms) taken; 2 samples were also taken from different parts of the cervix and uterine body and 4 samples were taken at regular intervals from non-caruncular portions of the pregnant horn and from the non-pregnant horn. Each sample was weighed, frozen and stored at −20°C until assay.

Collagen analysis

Hydroxyproline was estimated from 3–5 replicate analyses, in neutralised hydrolysates of tissue samples using the method of Grant (1964) with an autoanalyser system (ChemLab). The collagen concentration was calculated from the hydroxyproline value by taking the amino-acid content of collagen as 14% (w/w) (Etherington and Sims, 1981). Collagen contents were estimated from the collagen concentrations and the wet weight of the whole uterus and its different parts (uterine body, pregnant and non-pregnant horn and cervix).

Pyridinium crosslink assay

Pyridinium crosslinks (pyridinoline and deoxypyridinoline) were measured in hydrolysates from non-pregnant uterine tissues and from uterine tissues at term by high pressure liquid chromatography (HPLC) using the method of Black *et al* (1988).

RESULTS

Uterine weight

The change in weight of the different components of the uterus during pregnancy is shown in figure 1. The uterus at term was at least 10 times heavier than its non-gravid size. The wet weight increased from 1 100 ± 16.0 to 12 050 ± 1 560 g. Increases in dry weight (from 250 ± 20 to 2 600 ± 180 g) were equivalent and the ratio of dry/wet weight remained constant (about 21%) indicating there was no change in the proportion of water.

All the components of the genital tract increased in weight during pregnancy; the increase in weight of the pregnant horn was greatest. From mid-term, the caruncles represented the major component of the total weight of the pregnant uterus increasing from 71.25 ± 34.2 g at 2 months to 7 769 ± 2 436 g at the end of gestation. There was about an eightfold increase in the non-caruncular portion (from a minimum of 328.8 ± 50.05 to a maximum of 2 874 ± 881 g).

The increase in weight of the non-pregnant horn of the uterus was about fivefold (from a minimum of 302 ± 8.5 to 1663 ± 965 g). The non-pregnant horn of the uterus showed the greatest variation in change of weight because there was variable extension of the placenta from one horn into the other. The weight of the uterine body roughly doubled (from a minimum of 33.25 ± 4.02 to 77.3 ± 8.6 g) but remained a very small proportion of the total weight of the uterus; the small amounts do not appear in figure 1. The cervix also increased in weight by more than twofold (from 370 ± 71.8 to 1 068 ± 126 g).

Collagen concentration and content

Collagen contents and concentrations of the different parts of the uterus at different stages of gestation are shown in figures 2 and 3, respectively. Despite slight reductions in collagen concentrations in the different tissues there was an increase in collagen content in the bovine uterus during pregnancy. The changes in the different tissues reflect the increase in the total uterine mass (fig 1).

From 1 to 4 months of gestation, the cervix was the most collagenous part of the genital tract. Its collagen content then (from 52.3 to 77.77 g) was greater than that of the rest of the uterus (from 36.02 to 52.36 g). Between 5 and 9 months, uterine collagen exceeded that in the cervix increasing from 93.21 ± 15 to 358.45 ± 23.6 g in the uterus and from 79.39 ± 1.68 to 80.38 ± 12.72 g in the cervix. The greatest increase (about 16-fold) occurred in the pregnant horn, and the caruncles represented about half of the total. Roughly half the increase in uterine collagen occurred in the last trimester.

The collagen concentration of the uterine horns changed little, decreasing only slightly, in both non-caruncular portions of pregnant horns and non-pregnant horns, from 46.7 ± 2.1 and 51.8 ± 3.5 mg/g, respec-

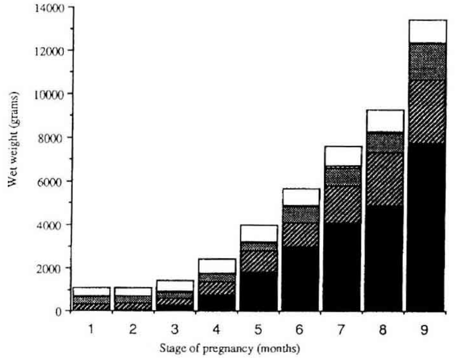

Fig 1. Change in weight of different parts of the genital tract of cattle during pregnancy. ▯ cervix; ▥ uterine body; ▩ non-pregnant horn; ▨ pregnant horn; ■ caruncles.

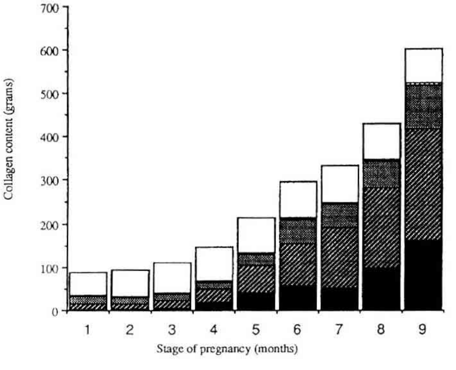

Fig 2. Change in collagen content of different parts of the genital tract of cattle during pregnancy. ▯ cervix; ▥ uterine body; ▩ non-pregnant horn; ▨ pregnant horn; ■ caruncles.

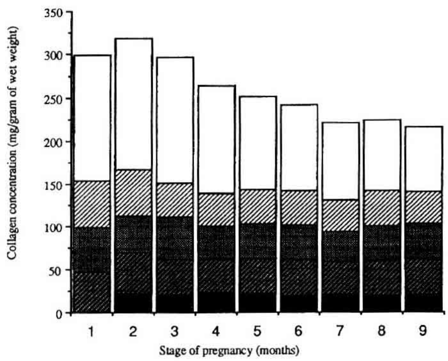

Fig 3. Change in collagen concentration in different parts of the genital tract of cattle during pregnancy. □ cervix; ▨ uterine body; ▩ non-pregnant horn; ▨ pregnant horn; ■ caruncles.

tively, at the beginning of gestation to 40.3 ± 2.2 and 41.3 ± 4.2 mg/g at the end of gestation. Collagen concentrations also decreased slightly in the uterine body (from 55.4 ± 9.6 to 37.6 ± 4.2 mg/g). There was a greater decrease in the cervix with collagen concentrations falling from 142 ± 16.5 mg/g at the beginning of gestation to 74.9 ± 3.2 mg/g at the end. Collagen concentration of caruncles changed little, being 22.8 ± 1.2 mg/g at 2 months of gestation and 20.9 ± 1.8 mg/g at term.

Pyridinium crosslinks

Non-pregnant uterine tissue contained 0.13 ± 0.04 ($n = 8$) residues of pyridinoline per mole of collagen. At term, the mean concentration of pyridinoline was identical (0.13 ± 0.05 ($n = 8$) residues/mole). The amounts of deoxypyridinoline, which is specific for bone collagen, were negligible (< 0.005 residues/mole). There were no systematic variations in crosslink content with different anatomical areas, although a slightly lower average pyridinoline concentration (0.09 residues/mol) was detected in the cervix.

DISCUSSION

The changes in connective tissue of the uterus and cervix during pregnancy and in the *post partum* period have been extensively studied as a model for those factors which may regulate the structure of the extracellular matrix. The subject has been reviewed recently by Jeffrey (1991). This is the first report of changes in collagen content of the bovine uterus during pregnancy and is similar to results obtained in other species such as rat (Harkness and Harkness, 1954) and human (Montford and Perez-Tamayo, 1961; Morrione and Seifter, 1962). In humans, collagen represents a major protein component of the uterus, cervix and foetal membranes; it constitutes up to 38% of total protein in the uterus and up to 80% in the cervix (Danforth and Buckingham, 1974). In the rat, the increase and decrease in the weight of the uterus is paralleled by deposition and resorption of collagen (Harkness and Harkness, 1954). The proportional increase in weight of the uterus of dairy cattle described here is essentially similar to that reported in beef heifers by Ferrel *et al* (1976), although the absolute weights were smaller in the younger animals used in that study. In both rats (Harkness and Harkness, 1954) and women (Morrione and Seifter, 1962) collagen increases continually from the first third of pregnancy. A similar time course was observed in the present study. This is similar in many species suggesting that the mechanism of control of collagen metabolism has been conserved during evolution (Jeffrey, 1991). In contrast to uterine collagen, changes in cervical collagen are more variable between species. In mice (Rimmer, 1973), rats (Harkness and Harkness, 1954), sheep (Fosang *et al,* 1984) and humans (Uldjberg *et al,* 1983) as well as in the cattle described here, the collagen content of the cervix increased during pregnancy. In the rabbit, however, there is virtually no change (Koob and Ryan, 1980).

Concentration is of greater relevance than amounts of collagen. In this report, and in other species, the concentration of collagen in the cervix increases during pregnancy.

The level of pyridinoline crosslinking reported here for bovine uterus is similar to that reported in human uterus (0.11 residues per mole) by Gunja-Smith and Woessner (1985). The increase in total uterine pyridinoline crosslinks, which must follow the increase in uterine collagen during pregnancy, supports the finding of Kaidi *et al* (1991) that *post partum* urinary excretion of pyridinoline reflects uterine collagen breakdown during involution. Pyridinoline is an important crosslink in stabilising collagen fibres. It occurs in the human uterus (Gunja-Smith and Woessner, 1985) but has not previously been demonstrated in bovine uterine tissues, although the increased urinary excretion of this compound during involution accounts for all the crosslinks lost from the uterus during degradation of collagen at this time (Kaidi *et al*, 1991). If pyridinoline is important for the mechanical strength of collagen then it is essential that the links form before labour, when the greatest mechanical forces will be placed on the collagen framework of the uterus. Biochemical changes in human cervical collagen associated with parturition suggest that they might play a significant role in cervical dilatation (Kleissl *et al*, 1978).

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