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Growth of Graafian Follicles in Cows following Luteolysis Induced by the Prostaglandin $F_{2\alpha}$ Analogue, Cloprostenol

R. J. Scaramuzzi, K. E. Turnbull and C. D. Nancarrow

Division of Animal Production, CSIRO, P.O. Box 239,
Blacktown, N.S.W. 2148.

Abstract

The ovaries of six multiparous cows 6–8 years old were collected 49–71 h after injection of a synthetic prostaglandin F analogue (cloprostenol) and serially sectioned. All follicles $>0{\cdot}4$ mm in diameter were counted and measured. The number of normal follicles ranged from 46 to 360 per cow. Atresia was not seen in follicles of $<1{\cdot}7$ mm diameter. The number of early atretic follicles ranged from 4 to 20 per cow.

The volume and mitotic index of the granulosa tissue was calculated for all follicles. Follicles were then grouped into classes according to granulosa volume, each class having twice the volume of granulosa of the preceding class. In all cows the rate of growth of follicles was slow up to $0{\cdot}5$ mm diameter (class 4). Above this size growth accelerated to a maximum in follicles of $1{\cdot}2$–$1{\cdot}7$ mm diameter (class 8) and declined thereafter to a minimum in follicles 8–10 mm in diameter (class 13). Follicles beyond class 13 were not present in any of the ovaries. It was estimated that $22{\cdot}1$ days were required for a follicle to grow from $0{\cdot}4$ mm to 10 mm in diameter (classes 4 through to 13).

The present data suggest that the pattern of synchronization of oestrus in cattle treated with cloprostenol may be related to the size of the largest non-atretic follicle present at the time of treatment because of the time required for such a follicle to complete its development.

Introduction

The synchronization of oestrus in cattle has been greatly facilitated by the use of exogenous luteolytic prostaglandins (Nancarrow and Cox 1976). However, the timing of oestrus and ovulation in relation to prostaglandin treatment is highly variable (Nancarrow and Cox 1976). These unpredictable responses mean that either oestrous detection or double insemination of a herd is necessary to achieve normal fertility (Nancarrow and Cox 1976). Following prostaglandin injection the time course of luteolysis is uniform (Cooper and Rowson 1975) indicating that variations in oestrous responses are primarily due to differences in the growth and development of mature Graafian follicles capable of ovulating.

In mammals, the development of follicles consists of an initial period of accelerated growth of small vesicular follicles followed by a subsequent period of differentiation during which follicles either become atretic and regress, or complete their maturation and ovulate at oestrus. Descriptive aspects of follicular development in the bovine ovary are well documented (Cole 1933; Asdell 1960; Rajakoski 1960; Hafez and Ishibashi 1964; Donaldson and Hansel 1965; Erikson 1966; Marion *et al.* 1968; Priedkalns *et al.* 1968; Dufour *et al.* 1972). However, quantitative data on the rate of follicular growth in the cow is not available and is clearly an essential to understanding the variability in the time of ovulation following surgical (Brock and Rowson

1952) or chemical (Cooper and Rowson 1975) destruction of the corpus luteum. The aim of this study was to quantitatively appraise ovarian follicular growth in cows treated with a luteolytic prostaglandin. The parameters selected for study were follicle number, follicle size, and the volume and mitotic index of the granulosa.

Materials and Methods

Six dry multiparous cows 6–8 years old were available for study. They were checked daily for signs of oestrus using Kamar heat-mount detectors (Kamar Inc., Steamboat Springs, Colorado, U.S.A.) until each cow had completed two normal oestrous cycles. A single intramuscular injection of 500 μg prostaglandin $F_{2\alpha}$ analogue (cloprostenol, I.C.I. (Aust.) Ltd, Melbourne) was administered during the early or late luteal phase of the oestrous cycle (Table 1). Three-hourly checks for oestrus were conducted from 24 h after cloprostenol injection up to ovariectomy, which was performed 49–71 h after treatment, the time over which cows must be in oestrus for a single, fixed-time insemination to be successful. A blood sample (100 ml) was taken by jugular venepuncture immediately prior to ovariectomy. Following centrifugation (15 min at 4°C; 1000g) the plasma was removed and the concentrations of oestradiol-17β (Scaramuzzi and Land 1978), progesterone (Thorneycroft and Stone 1972) and luteinizing hormone (LH) (Radford *et al.* 1978) measured by radioimmunoassays. The intra- and interassay coefficients of variation were 11·7 and 24·2%, 12·3 and 17·4%, 8·9 and 17·4% for oestradiol, progesterone and LH assays respectively. The minimum detectable amounts of hormone (i.e. zero standard minus two standard deviations of the zero standard) were 1·0, 10·0 and 50·0 pg per tube for oestradiol, progesterone and LH respectively.

Table 1. Treatment regime of cows and levels of circulating hormones

Treatment regime of six cows that had shown at least two normal oestrous cycles before the injection of cloprostenol (PG) together with the levels of circulating luteinizing hormone (LH), oestradiol-17β and progesterone at the time of ovariectomy

Cow No.	Breed	Day of cycle when PG given[A]	Time of oestrus after PG (h)	Time of ovary colln after PG (h)	LH (ng/ml)	Oestradiol-17β (pg/ml)	Progesterone (ng/ml)
71	Friesian	8	—	54	8·6	8·4	1·2
105	Hereford × Friesian	15	—	49	9·2	1·9	0·7
110	Angus × Friesian	16	48	50	4·6	1·9	1·0
63	Hereford × Friesian	16	—	51	9·0	3·6	0·5
76	Angus × Friesian	18	—	71	12·2	4·4	0·9
78	Angus × Friesian	19	48	53	5·1	1·4	1·0

[A] Oestrus = day 0.

Both ovaries from each cow were immersed in Serra's fixative for 24–48 h, stored in 70% ethanol, embedded in paraffin and serially sectioned at 15 μm. Every fifteenth section was mounted and stained with haematoxylin and eosin. A microprojector was used to locate sections in which follicles greater than 0·3 mm diameter attained their maximum size. Follicle diameter, volume and mitotic index (percentage of dividing cells) of the granulosa were determined by the method of Turnbull *et al.* (1977). Follicles were classified according to granulosa volume (GV) commencing with class 1, defined as having a GV of 2–4 $\times 10^{-6}$ μm^3 (Turnbull *et al.* 1977). Every fifteenth section (0·225 mm) was counted and consequently only follicles from class 4 (GV, 16–32 $\times 10^{-6}$ μm^3; diameter approximately 0·34 mm) and upwards were able to be measured accurately. The time required for

follicles to pass from one class to the next, that is the doubling time, was estimated from the formula (Hoffman 1949):

$$T = (\ln 2 \times t \times 100)/I,$$

where

T = doubling time;

t = mitotic time (0·43 h), the time required for granulosa cells of the sheep ovary to complete one mitotic division. A value for the cow ovary is not available; however, a similar time is required to complete a mitotic division in a variety of other mammalian tissues (i.e. 0·5 h) (Lushbough 1956; Hooper 1961); and

I = mitotic index of granulosa tissue.

Follicles were segregated into two categories, depending upon the presence or absence of atresia (Rajakoski 1960; Marion *et al.* 1968). Non-atretic follicles possessed a dividing granulosa although occasionally pycnotic nuclei as well as a few (less than 5) isolated 'atretic bodies' were also present. Early atretic follicles contained atretic bodies dispersed throughout the granulosa cells which also contained normal mitoses. Follicles in which atresia was more advanced, for example, where the granulosa still possessed an intact basement membrane but had a high incidence of atretic bodies and no mitoses or follicles in which the granulosa and oocyte were in a state of disintegration, were not considered.

Results

Six cows were treated with cloprostenol during the luteal phase of the oestrous cycle. Two were in oestrus by 48 h post-treatment and had ovulated by the time of ovariectomy, the remaining four had not shown oestrus and had not ovulated by the time of ovariectomy. The plasma concentrations of luteinizing hormone, oestradiol-17β and progesterone at the time of ovariectomy are shown in Table 1 with the low progesterone values indicating that luteolysis had taken place in response to the injection of cloprostenol. The four non-oestrous cows had either elevated LH or oestradiol-17β concentrations or both, indicating a probable pro-oestrous condition.

Table 2. Number of non-atretic (NA) and early-atretic (EA) follicles per follicle class for each cow examined

Cow No.	Follicle type	Follicle class 4	5	6	7	8	9	10	11	12	13	Total
71	NA	27	16	9	5	3	4	6	0	0	1[A]	70
	EA						1	4	0	0	0	5
105	NA	20	22	10	9	11	2	3	1	2	1	81
	EA						3	2	3	1	0	9
63	NA	50	35	29	18	5	5	6	5	0	1	154
	EA						1	3	7	0	0	11
76	NA	17	23	15	8	10	2	4	0	0	1	80
	EA						1	3	0	1	0	4
78[B]	NA	12	9	7	8	2	2	3	3	0	0	46
	EA						3	2	0	0	0	5
110[B]	NA	115	91	57	37	18	12	18	12	0	0	360
	EA						5	12	3	0	0	20

[A] Follicle ruptured during collection.

[B] Cow had ovulated by the time of ovariectomy.

Follicle Numbers

The number of non-atretic follicles and early-atretic follicles in each follicle class (4–13) was highly variable between animals (Table 2). One cow (No. 110) although apparently normal had a much larger population of follicles than the other five.

The absence of class 13 non-atretic follicles in the cows which had ovulated (Nos. 78 and 110) was no doubt due to that fact. The four remaining cows each had a class 13 non-atretic (i.e. preovulatory) follicle at ovariectomy. Atresia was first encountered in class 9 follicles (i.e. at least 1·7 mm diameter); however, the granulosa of some class 8 follicles though classed as non-atretic already showed signs of developing atresia. Both the incidence and pattern of atresia was similar in all cows (Table 2). An estimate of the mean number of follicles per day entering the phase of rapid growth (i.e. classes 5–12) was obtained from the number of transitory class 5–8 follicles and the time required for growth through these classes. This is possible because there is no loss through atresia in follicles until they reach class 9. Thus defined, there were uptake rates of 6·2 follicles per day in the five cows with similar follicle numbers and 26·9 in the remaining cow (No. 110).

Follicle Growth

The growth of the bovine Graafian follicle 49–71 h after cloprostenol treatment followed a well-defined pattern (Table 3). Growth was slow in small vesicular follicles (class 4) and increased rapidly with increased diameter (classes 5–8), reaching a maximum in class 8 follicles and then decreasing slowly to about half the maximum value in class 11 follicles. Despite our low numbers it is likely that this reduced rate of growth was maintained in larger follicles (classes 12 and 13). The mean overall time required for follicles to grow through classes 4–13 is 531·3 h or 22·1 days.

Table 3. Follicle number, follicle diameter, volume and mitotic index of the granulosa and follicle doubling times of non-atretic follicles from six cows treated with cloprostenol

Values given are means±s.e.m.

Follicle class[A]	No. of follicles measured	Follicle diameter (μm)	Granulosa volume $\times 10^{-6}$ (μm^3)	Mitotic index of granulosa	Estimated doubling time[B] (h)
4	190	409±5	22·5±0·5	0·37±0·02	80·5
5	154	560±7	45·1±0·9	0·45±0·03	66·2
6	111	745±11	84·5±2·4	0·60±0·04	49·7
7	73	1025±19	177±6	0·82±0·04	36·3
8	47	1450±33	371±13	1·02±0·05	29·2
9	27	2045±54	816±37	0·91±0·05	32·7
10	40	2954±72	1484±61	0·77±0·05	38·7
11	19	3792±102	2534± 123	0·63±0·06	47·3
12	2	5564±324	4659± 220	0·58±0·13	51·4
13	3	8919±528	14179±1228	0·30±0·14	99·3

[A] Follicle classes 5–12 in phase of rapid growth.
[B] Based on mitotic time of 0·43 h derived from the granulosa of sheep (Turnbull *et al.* 1977).

Discussion

There is general agreement that bovine Graafian follicles grow and regress continuously throughout the oestrous cycle and that there is no evidence to suggest the presence of 'resting stages' during the vesicular phase of their development (Rajakoski 1960; Donaldson and Hansel 1965; Erikson 1966; Priedkalns *et al.* 1968). Follicles can attain a diameter of about 10 ± 2 mm independently of day of cycle (Marion *et al.* 1968) and those destined to ovulate rapidly increase in size (through fluid

accumulation) to about 15–18 mm diameter during pro-oestrus and oestrus (Dufour *et al.* 1972). The present findings are consistent with this general view and indicate that the time required for Graafian follicles to grow from about 0·4 mm to about 10·0 mm diameter (classes 4 through to 13) is approximately 22 days. Class 13 was the largest follicle class present and probably is the preovulatory class, the final preovulatory swelling to 15–18 mm diameter resulting from fluid accumulation and not from cell division.

The pattern of follicle growth in cloprostenol-treated cows (Table 3) is similar to that seen in sheep during the oestrous cycle (Turnbull *et al.* 1977) and the growth curves for each are compared in Fig. 1. This comparison, although not controlled,

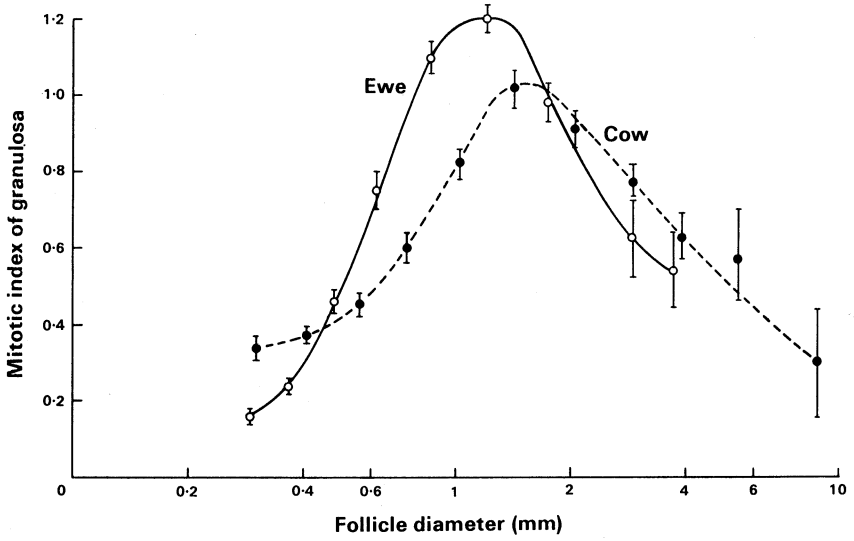

Fig. 1. Change in the mitotic index (mean ± s.e.m.) of the granulosa with growth of the Graafian follicle in six cows treated with cloprostenol (●) and 17 Merino sheep (○). Data for Merino sheep adapted from Turnbull *et al.* (1977).

highlights differences which we believe to be important in understanding the degree of oestrous synchronization following cloprostenol. The maximum growth rate of the granulosa in cow follicles is attained in bigger follicles (1·5 mm diam.: class 8) than in sheep (0·10 mm diam.: class 7). Likewise the size at which follicles can luteinize is probably larger in the cow (8·0 mm diam.: Marion *et al.* 1968) than in the sheep (3·5 mm diam.: Smeaton and Robertson 1971). These results suggest that Graafian follicles in cows need to persist longer and grow to a greater size (class 13) than those in sheep (class 11) before they are able to respond to luteinizing hormone and ovulate. The variations in time between prostaglandin treatment and the onset of oestrus and ovulation could be related to the time required for the largest follicle present at treatment to complete its growth and maturation to the preovulatory stage. This suggestion is consistent with the reported variations in the degree of oestrous synchrony in sheep and cattle treated with prostaglandins (Hearnshaw *et al.* 1974; Nancarrow and Cox 1976).

The availability of non-atretic class 13 follicles in the cow could also be an important factor in determining the time delay between prostaglandin treatment and

oestrus. The number of non-atretic follicles >1·0 mm diameter is rapidly reduced by atresia (Marion *et al.* 1968). The incidence of atresia in sexually mature heifers increases with follicle number effectively restricting the number of non-atretic follicles >5·0 mm diameter to 1·5 per cow regardless of the total number of follicles (Rajakoski 1960). Similarly our results indicate an average of 0·7 (4/6) class 13 follicles per cow and 0·3 class 11 follicles per sheep (Turnbull *et al.* 1978) are present at any one time. Based on these calculations the availability of mature non-atretic follicles in cattle and in sheep is similar, being about 2–3 per oestrous cycle; this number is probably insufficient to ensure a constant supply of preovulatory follicles.

The results of these preliminary investigations suggest that an important factor affecting the degree of synchronization of oestrus following treatment with prostaglandin is probably the size of the largest follicle present at the time of treatment and the time required for that follicle to complete its development to the preovulatory stage.

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