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Relationship between ovarian Δ^5-3β-hydroxysteroid dehydrogenase activity and implantation in non-lactating and lactating Mongolian gerbils *(Meriones unguiculatus)*

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Summary. The distribution and intensity of Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD) activity was examined histochemically in ovaries from Mongolian gerbils during pregnancy and undergoing lactational delay. In all of the ovaries examined the corpora lutea showed the strongest level of activity, but like the interstitium and granulosa, there was no relationship between the enzyme pattern and embryonic development. Thecal 3β-HSD activity was only observed immediately before, and after implantation on day 8 p.c. in pregnancies without lactation, and was totally absent throughout lactational delay, appearing for the first time after day 15 in association with implantation. The absence of this key enzyme in the theca interna throughout the pre-implantation period means that the biosynthesis of androstenedione and progesterone cannot occur, thereby precluding the production of oestrogen, an essential steroid for implantation in this species.

Introduction.

In the non-lactating, post parturient Mongolian gerbil implantation occurs on day 8 (Norris and Adams, 1971 ; Fischer and Floyd, 1972), but in the presence of concurrent lactation implantation is delayed according to the number of young suckled (Norris and Adams, 1981).

It has been clearly demonstrated that ovarian progesterone and oestrogen are required for implantation in pregnancies with or without concurrent lactation (Wu, 1975 ; Norris, 1979), and a continued dependency on these ovarian steroids for successful post-implantation development is indicated by the demonstration that ovariectomy on the 18th day of pregnancy causes abortion within 24 h (Marston and Chang, 1965). Studies on antral follicles (Norris and Adams, 1971) and corpora lutea (Meckley and Ginther, 1972 ; Bagwell, 1977) have indicated points of significant ovarian change in relation to embryonic development, without giving any information on the sites of steroid synthesis within the ovary.

The enzyme Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD) is essential in the biosynthesis of ovarian steroids by catalysing the conversion of both C_{21} and C_{19} steroids. It effects the conversion of pregnenolone to progesterone and dehydroepiandrosterone to androstenedione, and thereby controls the ultimate production of ovarian oestrogen. Since Wattenberg (1958) clearly demonstrated the experimental visualization of this enzyme by formazan deposition of an introduced tetrazolium salt, the technique has been successfully used to examine ovarian activity in a wide range of species (Albrecht, Koos and Wehrenberg, 1975 ; Blaha and Leavitt, 1970 ; Hay and Moor, 1975 ; Hay, Allen and Lewis, 1975 ; Pupkin *et al.,* 1966). The following report examines the presence and activity level of 3β-HSD in the ovaries of non-lactating and lactating, pregnant gerbils.

Materials and methods.

Animals. — A total of 40 mature females from the colony at the Animal Research Station, Huntingdon Road, Cambridge were maintained as described earlier (Norris and Adams, 1981), and used following the confirmation of post partum mating (= day 1 *post coitum* (p.c.)). Litters were then either removed (N = 20) or adjusted to 5-6 young (N = 20), this number being maintained by cross-fostering whenever necessary. These females were used at intervals from day 3-25 p.c.

Autopsy procedure. — Non-lactating females were autopsied on day 3-10 inclusive and days 15 and 20. The lactating females were autopsied on day 3-7 inclusive and on days 10, 15, 20 and 25. In both groups, 2 females were autopsied at each stage p.c., excepting for the lactating group on day 20 when 4 females were used. Reproductive tracts and ovaries were removed and the ovaries (1 per female) were immediately dissected for histochemistry (see below). Oviducts and uteri were then dissected, and in the absence of uterine implantations, the oviducts were placed in a punted watch glass containing phosphate-buffered saline (PBS) and opened using fine needles. Each uterine horn was flushed with PBS into a punted watch glass. Oviducal macerates and uterine flushings were then searched for eggs using a stereomicroscope.

Histochemistry. — The freshly dissected ovaries were rapidly embedded in O.C.T. compound (Ames Co., Indiana, USA) surrounded by solid CO_2 and stored at − 20 °C until cutting. Ovaries removed from females having normal eggs recovered, or implantations present, were subjected to an experimental procedure based on that described by Wattenberg (1958), Hay and Deane (1966) and Hay and Moor (1975). Blocks were cut in a cryostat, 4 central ovarian sections being taken. Lipids and endogenous substrate were removed by cold acetone and PBS wash before incubating the sections at 37 °C for 4 h. Four incubations were carried out simultaneously on each ovary ; dehydroepiandrosterone (DHA) (Steraloids Ltd., Croydon) + nicotinamide adenine dinucleotide (NAD) (Boehringer Corporation London Ltd., Lewes, Sussex) for 3 and 4 h being the experimental groups, and 2 controls ; one having NAD without the substrate, and

a second with NADH (Boehringer Corporation London Ltd., Lewes, Sussex) to test for the presence of diaphorase activity. Nitro-blue tetrazolium (NBT) (Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey) was used in all incubations.

After incubation, sections were fixed, washed and mounted in Hydramount (Searle Diagnostic, High Wycombe, Bucks) and examined microscopically for formazan deposition. The experimental sections were scored subjectively on a 0 to 4 scale (0 = absent, 1 = weak, 2 = moderate, 3 = strong and 4 = very strong).

Results.

Embryonic development. — In the absence of lactation, eggs were recovered from the oviducts as morulae or early blastocysts on day 5, with all of the eggs recovered from the uteri as blastocysts on day 6. Implantation had occurred by day 8. In the presence of lactation, development was similar to that found in non-lactating females up to day 5, but of the eggs recovered on day 6, 29 % were recovered from the oviducts and 30 % were still morulae. From day 7 to 15 blastocysts were recovered from the uteri of all the lactating females examined. Of the 4 lactating females examined on day 20, 2 had implantations and 2 had blastocysts present. Both of the females examined on day 25 had implantations present.

Ovarian 3β-HSD activity. — The two controls gave the same results in all incubations, ones with only NAD being negative, and the ones with NADH being positive. The levels of 3β-HSD activity within the various ovarian compartments following incubation of the experimental sections are given in Table 1.

TABLE 1

Intensity of ovarian Δ^5-3β-hydroxysteroid dehydrogenase activity in non-lactating (NL) *and lactating* (L) *gerbils during post partum pregnancy.*
Activity assessed in 40 ovaries from 40 females using a 0 (absent) to 4 (very strong) scale.

	(NL)				(L)			
Days p.c.	Ovarian 3β-HSD activity							
	Corpora lutea	Interstitium	Theca interna	Granulosa	Corpora lutea	Interstitium	Theca interna	Granulosa
3	2, 3,	2, 2,	0, 0,	0, 0.	3, 3,	2, 2,	0, 0,	0, 0.
4	2, 3,	1, 2,	0, 0,	0, 1.	3, 3,	1, 2,	0, 0,	0, 0.
5	3, 3,	1, 1,	0, 0,	0, 0.	2, 3,	0, 1,	0, 0,	0, 0.
6	2, 3,	1, 1,	0, 1,	0, 0.	2, 3,	0, 1,	0, 0,	0, 0.
7	3, 3,	1, 2,	1, 2,	1, 1.	2, 2,	1, 1,	0, 0,	0, 0.
8	2, 3	1, 2,	1, 2,	0, 1.	—	—	—	—
9	3, 4,	2, 2,	1, 1,	0, 0.	—	—	—	—
10	3, 3,	1, 1,	1, 1,	0, 0.	3, 4,	0, 1,	0, 0,	0, 0.
15	3, 3,	1, 2,	3, 3,	0, 1.	3, 4,	0, 1,	0, 0,	0, 0.
20(a)	—	—	—	—	3, 3,	0, 0,	0, 0,	0, 0.
(b)	4, 4,	1, 1,	1, 1,	0, 1.	3, 3,	1, 1,	1, 1,	0, 1.
25	—	—	—	—	3, 4,	1, 1,	1, 2,	0, 1,

Broken line indicates the stage of implantation.

Corpora lutea. — All of the corpora lutea showed positive activity irrespective of the stage of pregnancy or whether or not the female was lactating. At every stage examined the corpora lutea were the most strongly reactive component (see Plate 1, A, B, D & E). No activity pattern was shown that could be related to the stage of pregnancy, and lactation did not appear to exert any influence.

Interstitium. — In the absence of lactation, the interstitium showed weak to moderate activity at all stages of pregnancy (Plate 1, A, E). In the lactating females activity was weak or moderate in a high proportion (70 %) of the ovaries examined, and absent in the rest (Plate 1, D).

Theca interna. — The theca was devoid of 3β-HSD activity in all ovaries from non-lactating females up to day 6, and present in all ovaries from day 7 onwards (Plate 1, A, C, E). The ovaries of lactating females had no thecal 3β-HSD activity up to and including day 15 (Plate 1, B, D). On day 20, lactating females were without thecal activity in association with blastocysts, and showed positive activity when implantations were present. Both of the lactating females autopsied on day 25 had implantations and positive thecal activity (Plate 1, F).

Granulosa. — The granulosa was the least reactive part of the ovary, being only weakly active in 30 % of the ovaries from non-lactating females, without any obvious association with implantation. Only 10 % of the ovaries from the lactating females showed any activity.

Discussion.

Up to day 5, a similar rate of egg development was found in both non-lactating and lactating females. However, after this stage lactation reduced the rate of development and transport. This is associated with a reduction in the rate

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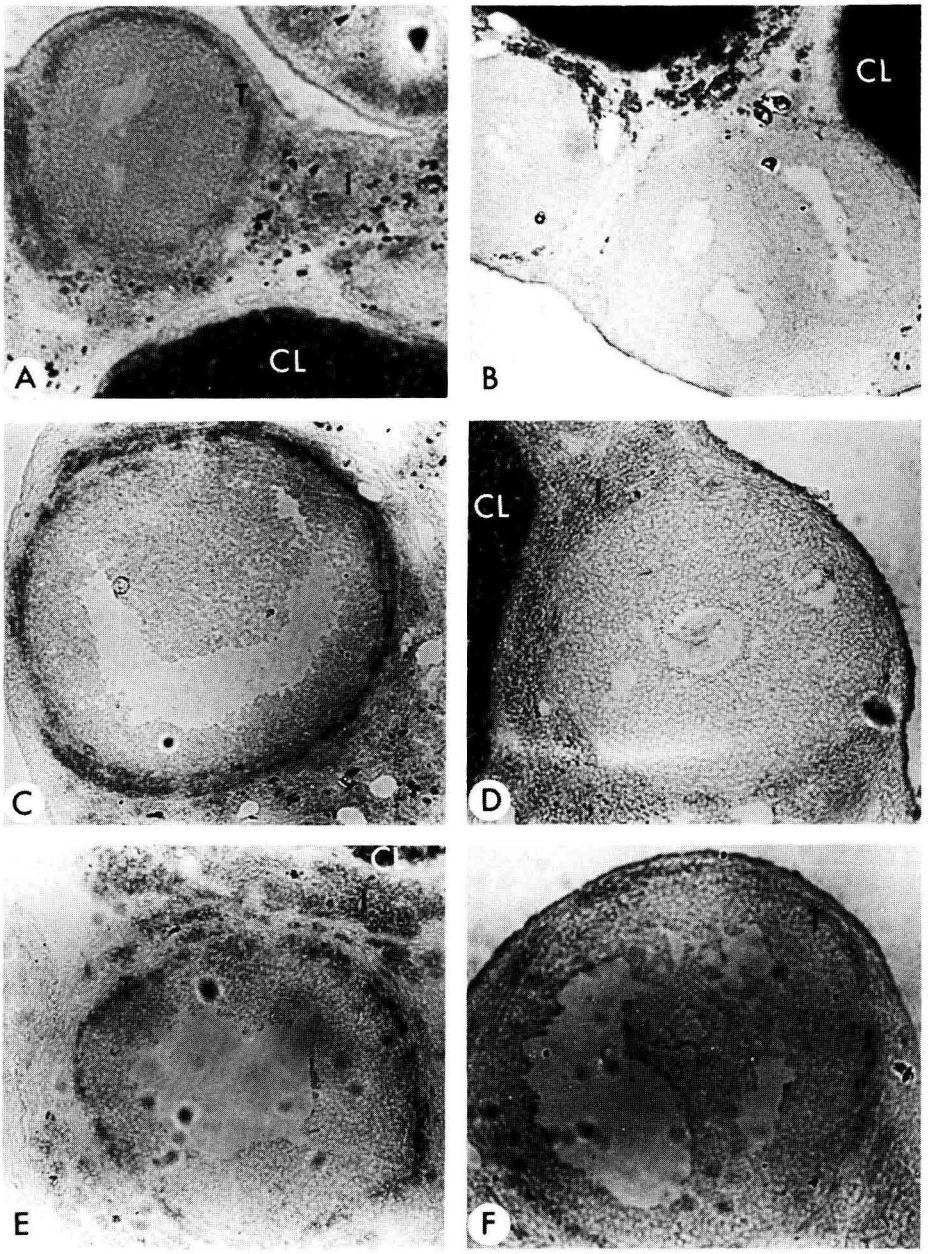
PLATE 1

Ovarian Δ^5-3β-hydroxysteroid dehydrogenase activity in non lactating (NL) *and lactating* (L) *pregnant gerbils.*

All sections were incubated with dehydroepiandrosterone + nicotinamide adenine dinucleotide (oxidized) + nitro-blue tetrazolium.
Abreviations : CL = corpus luteum, I = interstitium,
G = membrana granulosa, T = theca interna.

A. Day 10 (NL). Stong luteal activity, weak thecal and interstitial activity, no granulosal activity. Implants present. (x55).
B. Day 10 (L). Very strong luteal activity, no thecal, interstitial or granulosal activity. Blastocysts recovered. (x55).
C. Day 15 (NL). Strong thecal activity. Implants present. (x80).
D. Day 15 (L). Strong luteal activity, weak interstitial, no thecal or granulosal activity. Blastocysts recovered. (x90).
E. Day 15 (NL). Strong luteal and thecal activity, moderate interstitial activity. Implants present. (x55).
F. Day 25 (L.) Weak thecal activity. Implants present. Estimated = day 11 (NL) pregnancy. (x85).


T
I
CL
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F

of blastomere proliferation, which reaches significance by day 7 (Norris, 1979). Delayed entry of eggs into the uterus of lactating gerbils is in accord with an earlier suggestion by Bindon (1969) that lactation might delay egg transport in the mouse. With the rate of egg transport being influenced by steroid hormones, the present results correlate very well with the already noted ovarian changes associated with concurrent lactation (Norris and Adams, 1971 ; Norris, 1979).

The present observations on the incidence and intensity of luteal 3β-HSD activity do not indicate any points of significant change relating to embryonic development. Throughout, the uniformly positive, strong reaction for this enzyme indicates that the corpora lutea retain the potential for converting pregnenolone to progesterone throughout all stages of pregnancy, with or without concurrent lactation. To the author's knowledge, no progesterone estimations have been made for the pregnant gerbil, and the most useful information available consists of volumetric measurements with associated histological changes (Norris and Adams, 1971), and an ultrastructural study of corpora lutea (Bagwell, 1977). These studies showed a uniform increase in the size of the corpora lutea during the first 8 days of normal pregnancy, which together with increased lutein cell size and agranular cytoplasmic reticulum, strongly implies an increased output of progesterone during this period. However, throughout lactational delay the corpora lutea remain at a stage of development equivalent to Day 5, thereby indicating a period of constant, low level steroidogenic activity within the corpora lutea.

The present results showing a generally constant level of 3β-HSD activity in the interstitium of all of the ovaries from non-lactating females and in the majority (70 %) of the ovaries from lactating females is difficult to interpret. A constant interstitial activity occurring at the same intensity during all stages of the oestrous cycle, pregnancy and lactation has been reported for the hamster by Blaha and Leavitt (1970).

Within the gerbil follicles examined, the granulosa 3β-HSD activity was very spasmodic, weak and without any apparent association with embryonic events, whereas positive activity in the theca interna was closely related to implantation. There are conflicting reports relating to granulosa activity in the rat. Deane *et al.* (1961) found positive activity present in early atretic follicles only, whereas Pupkin *et al.* (1966) found activity present only in mature non-atretic follicles. In general it seems that 3β-HSD activity has been most commonly found in granulosa of atretic follicles of mouse, rat and hamster (see Baillie, Ferguson and Hart, 1966), and in the present study the spasmodic granulosa activity is considered due to either follicular atresia, or a ' false ' reading.

The present results on thecal 3β-HSD activity indicate a close association with implantation, and a notable absence throughout lactational delay. Earlier results on the gerbil showed that follicular maturation is suppressed throughout lactational delay, with a sharp increase in both the number and development of antral follicles at the time of implantation (Norris and Adams, 1971 ; Norris, 1979). In the rat a positive relationship is believed to exist between FSH, follicular maturation with a stimulated thecal layer and oestrogen secretion (Nishizuka, 1954 ; Raud, 1974 ; Nakano *et al.,* 1977). In the hamster too, a positive

correlation has been demonstrated between the cyclic development of antral follicles, the appearance of the necessary enzymes and a rise in the circulating and ovarian oestrogen levels (Greenwald, 1961 ; Shaikh, 1972 ; Baranczuk and Greenwald, 1973 ; Saidapur and Greenwald, 1978). In lactating, pregnant gerbils a significant increase in both ovarian weight and peripheral oestrogen was found 20 h following litter removal (Norris, 1979), these events are believed to be largely due to follicular maturation following the removal of lactational suppression. Both *in vivo* and *in vitro* studies on sheep follicles have demonstrated a positive correlation between 3β-HSD activity and oestrogen output (Hay and Moor, 1975). A two-cell theory was originally proposed by Falck (1959) for the rat, with the thecal cells actively synthesizing androgens and the granulosa cells being essential for aromatisation of androgens to oestrogen. This concept is now more generally accepted, having been demonstrated for several other species including the hamster (Makris and Ryan, 1975).

The present histochemical results on the gerbil ovary accord with the two-cell theory, with the presence of 3β-HSD activity in the theca interna being an essential, initial requirement for the ultimate production of follicular oestrogen. The activity of this enzyme is in turn dependent on the follicle having obtained sufficient maturity. Follicular maturation is under pituitary gonadotrophin control, the secretion of which is suppressed by intense lactation concurrent with pregnancy, resulting in an absence of ovarian oestrogenic activity and delayed implantation.

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Résumé. *Relation entre l'activité Δ^5-3β-hydroxystéroïde deshydrogenase ovarienne et l'implantation chez la Gerbille de Mongolie (Meriones unguiculatus) lactante ou non lactante.*

La distribution et l'activité Δ^5-3β-hydroxystéroïde déshydrogenase ont été suivies par étude histochimique des ovaires de la gerbille de Mongolie au cours de la gestation et pendant la période d'implantation différée. Une forte activité a été observée dans les corps jaunes de tous les ovaires examinés ; mais, comme pour le tissu interstitiel ou la granulosa, aucune relation n'a été trouvée entre activité enzymatique et développement embryonnaire. Une activité 3β-HSD a été mise en évidence juste avant et après l'implantation au 8^e jour post coitum chez les femelles gestantes non lactantes, mais était totalement absente au cours du retard induit par la lactation ; elle apparaissait pour la première fois après le jour 15, au moment de l'implantation. L'absence de cette enzyme clé dans la thèque interne pendant la période précédant l'implantation signifie que la biosynthèse de l'androstènedione et de la progestérone ne peut avoir lieu, ce qui prévient la production d'estrogènes, stéroïde nécessaire à l'implantation dans cette espèce.

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