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Histochemical studies of Δ^5-3β-, 20α- and 20β-hydroxysteroid dehydrogenases and possible progestagen production in hamster eggs

S. Niimura and K. Ishida

Department of Animal Husbandry, Faculty of Agriculture, Niigata University, 2 Igarashi, Niigata, Japan

Summary. Hamster eggs at various developmental stages were studied histochemically for the presence of Δ^5-3β-hydroxysteroid dehydrogenase (HSD) (pregnenolone as the substrate and NAD as the cofactor), 20α-HSD (20α-hydroxypregn-4-en-3-one and NADP) and 20β-HSD (20β-hydroxypregn-4-en-3-one and NAD). All three enzymes were absent from ovarian eggs but were demonstrated in unfertilized and fertilized eggs up to the blastocyst stage. The activities varied only slightly.

Introduction

The steroid dehydrogenases, Δ^5-3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase, have been demonstrated histochemically in the eggs of rats, hamsters and rabbits using dehydroepiandrosterone and oestradiol-17β respectively as the substrates (Dickmann & Dey, 1973, 1974a, b; Dickmann & Gupta, 1974; Dey & Dickmann, 1974; Dickmann, Dey & Gupta, 1975a, b). The enzyme activities were said to appear for the first time in cleaved eggs at the time of transformation into blastocysts, and it was postulated that the eggs at this stage acquire an ability to produce steroid hormones which might play some role in the formation of blastocysts. Pig blastocysts have been shown to contain various steroid dehydrogenases (Flood, 1974), and to be able to produce steroids *in vitro* (Perry, Heap & Amoroso, 1973).

In the ovary, progesterone is synthesized from cholesterol via pregnenolone, and is converted into 20α-hydroxypregn-4-en-3-one in the rat (Wiest, 1959), mouse (Loutfi, Péron & Dorfman, 1962) and mare (Short, 1964), and into 20β-hydroxypregn-4-en-3-one in the cow (Gomes & Erb, 1965). This investigation, therefore, was designed to demonstrate histochemically whether the enzymes that control these conversions are present in ovarian and tubal hamster eggs at various developmental stages, and to explore the possibility of progestagen production by the eggs.

Materials and Methods

Sixty adult hamsters (*Mesocricetus auratus*) were used. The oestrous cycle was checked by examination of the post-oestrous discharge (Orsini, 1961), and only animals which became oestrous at about 18.00 hours were used. Since most hamsters ovulate 8 hr after the onset of oestrus (Harvey, Yanagimachi & Chang, 1961), the ages of eggs were determined by reference to the onset of oestrus. For follicular eggs, ovaries were removed 2 hr before ovulation, and cut serially at 15 μm using a cryostat. For the recovery of tubal eggs, females were mated with fertile males, and killed 2 hr after ovulation for unfertilized eggs, after 4 hr for penetrated eggs, 32 hr for 2-cell eggs, 52 hr for 4-cell eggs, 65 hr for 8-cell eggs and 76 hr for blastocysts; a few degenerating eggs were obtained when 2- and 8-cell eggs were collected. One- to 4-cell eggs were recovered by tearing the excised oviducts, immersed in 0·1 M-phosphate-buffer solution (pH 7·5) in a watch glass, under a stereoscopic microscope. Unfertilized and penetrated eggs thus obtained were immediately treated with 0·1% hyaluronidase to dissolve the cumulus oophorus, and then were transferred into fresh buffered solution.

Eight-cell eggs and blastocysts were recovered by flushing the uterine horns with the same buffered solution.

The three enzymes investigated were: Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) using pregnenolone as the substrate and NAD as the cofactor, 20α-hydroxysteroid dehydrogenase (20α-HSD) using 20α-hydroxypregn-4-en-3-one and NADP, and 20β-hydroxysteroid dehydrogenase (20β-HSD) using 20β-hydroxypregn-4-en-3-one and NAD respectively. To detect these enzymes a slight modification of the method used by Dickmann & Dey (1973) was employed: several sets of eggs from every developmental stage were placed into a solution containing 1·8 mg substrate (Sigma Co.) which had been dissolved in 0·5 ml acetone, 4·0 mg cofactor (Sigma Co.), 2·0 mg nitro-blue tetrazolium (Sigma Co.), and 10 ml 0·1 M-phosphate-buffer solution at pH 7·5. Eggs incubated in a medium containing the substrate solvent, acetone, but devoid of the substrates and NADPH were observed as controls. The incubation time was exactly 40 min, because this was the period within which unspecified or endogenous dehydrogenase reaction never appeared in control eggs incubated in substrate-free medium: an incubation time longer than 40 min resulted in the appearance of non-specific reaction even in control eggs. After incubation in one of the three substrate solutions for 40 min at 37°C, ovarian sections containing eggs were fixed in 10% formalin solution, and then mounted on slides with glycerin jelly. Tubal eggs were transferred into the buffered solution and then a drop of the solution including the eggs was placed in the centre of 4 Vaseline spots on a slide. A coverslip was then carefully placed on the Vaseline spots and gently depressed. Formalin solution (10%) was run under the edges of the coverslip as fixative and the morphological features of the eggs were examined under a phase-contrast microscope.

Results

Ovarian eggs

Several well-developed Graafian follicles containing mature eggs were present in the ovaries 2 hr before ovulation. The eggs were surrounded by the cumulus oophorus, and their nuclei were at metaphase, situated at the periphery of the cytoplasm; release of the first polar body had not occurred. No enzyme activities were observed in any of the ovarian eggs.

Tubal eggs

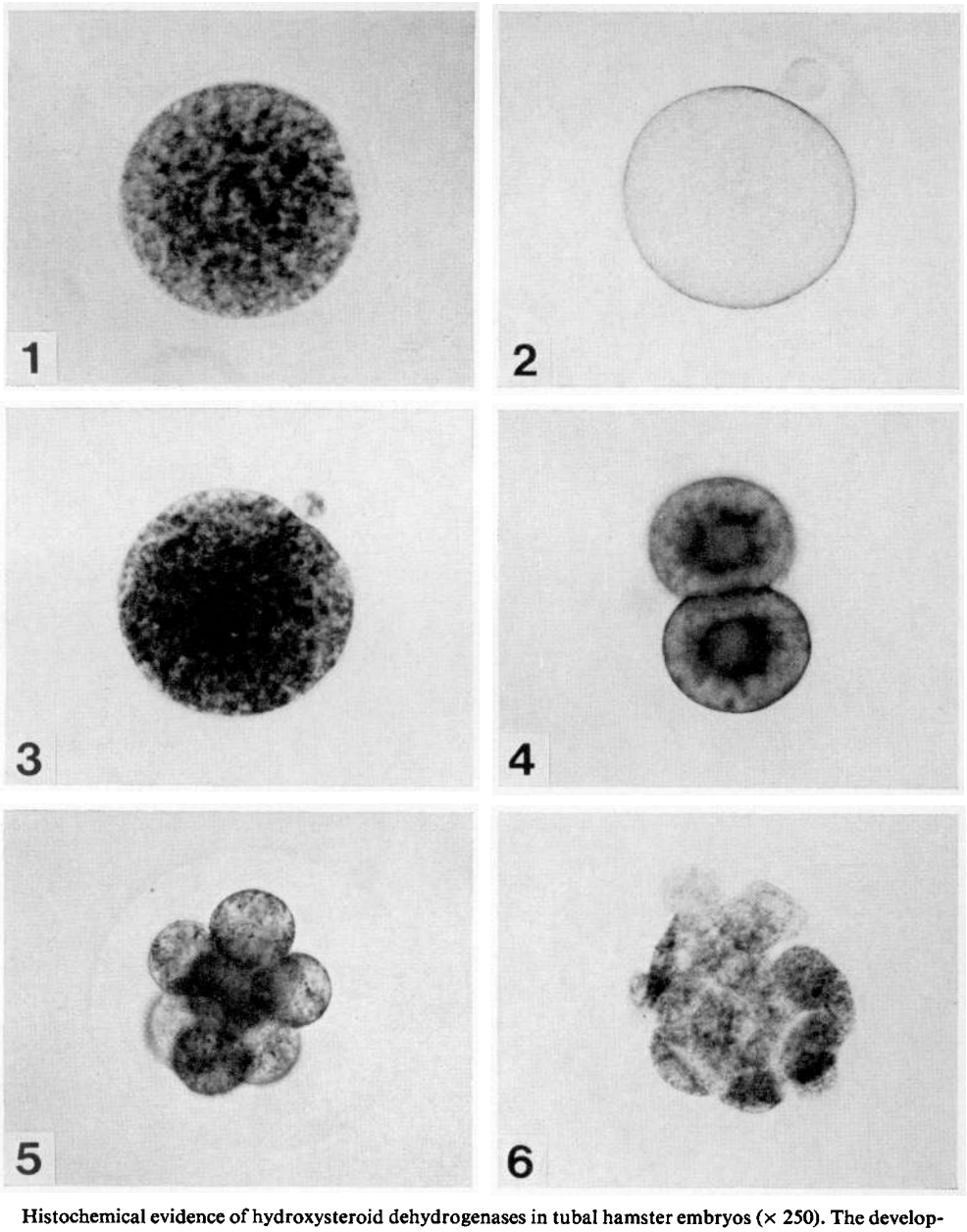
With all three substrates, tubal fertilized and unfertilized eggs showed evenly distributed diformazan granules in the cytoplasm (Pl. 1, Figs 1 and 3): no such granules were observed in the eggs immersed in substrate-free medium (Pl. 1, Fig. 2), indicating the presence of some Δ^5-3β-HSD, 20α-HSD and 20β-HSD activity. Some diformazan granules were present in the polar bodies. After cleavage, diformazan granules were found in the cytoplasm of the blastomeres, and were

Table 1. Hydroxysteroid dehydrogenase activities in hamster eggs

Enzymes	Substrates	Ovarian eggs*	Tubal eggs					
			Unfertilized	Fertilized				
				1-Cell	2-Cell	4-Cell	8-Cell	Blastocysts
Δ^5-3β-HSD	Pregnenolone	—	+++	+++	++	±	+	+
20α-HSD	20α-Hydroxypregn-4-en-3-one	—	+++	+++	++	++	++	++
20β-HSD	20β-Hydroxypregn-4-en-3-one	—	+++	+++	+++	+++	+++	++

—, Negative; ±, slight; +, weak; ++, moderate; +++, strong.
* Eggs in primordial, primary, secondary and Graafian follicles.

PLATE 1

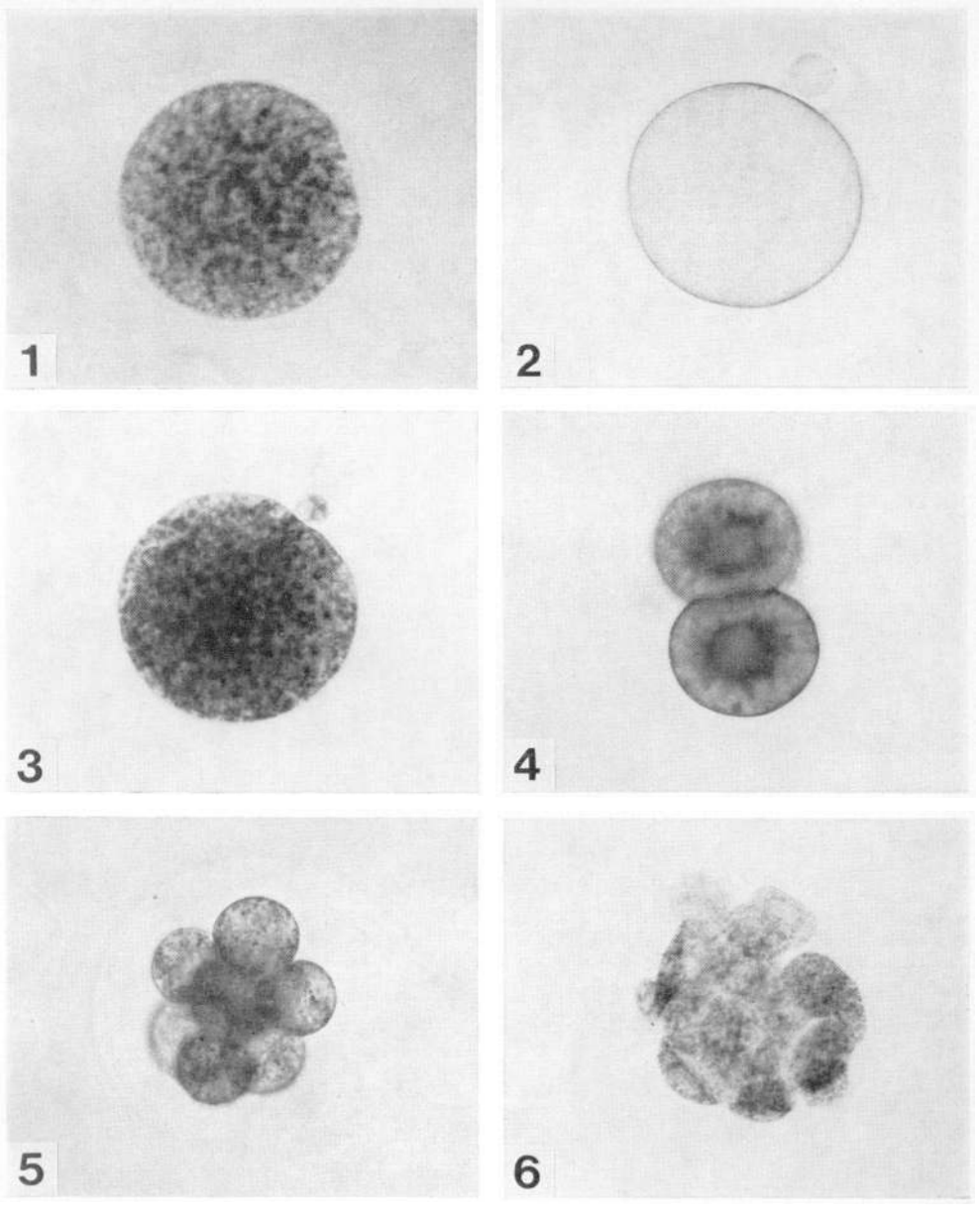


Histochemical evidence of hydroxysteroid dehydrogenases in tubal hamster embryos (× 250). The developmental stages and substrates were as follows.

Fig. 1. An unfertilized egg, 20α-HSD reaction, 20α-hydroxypregn-4-en-3-one.
Fig. 2. A penetrated egg, substrate-free medium.
Fig. 3. A penetrated egg, 20β-HSD reaction, 20β-hydroxypregn-4-en-3-one.
Fig. 4. A 2-cell egg, Δ^5-3β-HSD reaction, pregnenolone.
Fig. 5. An 8-cell egg, Δ^5-3β-HSD reaction, pregnenolone.
Fig. 6. A blastocyst, 20α-HSD reaction, 20α-hydroxypregn-4-en-3-one.

(*Facing p.* 276)

PLATE 1



Histochemical evidence of hydroxysteroid dehydrogenases in tubal hamster embryos (× 250). The developmental stages and substrates were as follows.

Fig. 1. An unfertilized egg, 20α-HSD reaction, 20α-hydroxypregn-4-en-3-one.
Fig. 2. A penetrated egg, substrate-free medium.
Fig. 3. A penetrated egg, 20β-HSD reaction, 20β-hydroxypregn-4-en-3-one.
Fig. 4. A 2-cell egg, Δ^5-3β-HSD reaction, pregnenolone.
Fig. 5. An 8-cell egg, Δ^5-3β-HSD reaction, pregnenolone.
Fig. 6. A blastocyst, 20α-HSD reaction, 20α-hydroxypregn-4-en-3-one.

(*Facing p.* 276)

especially abundant at the perinuclear region and the periphery of the cytoplasm (Pl. 1, Figs 4 and 5). There was no difference in the amount of granules in the two cell types, inner cell mass and trophoblast, of the blastocyst (Pl. 1, Fig. 6). Granules were never observed in the zona pellucida. The distribution pattern of diformazan granules as described above did not differ for the three enzymes. The changes of enzyme activity were judged by the amount of diformazan granules, which indicates the strength of activity and the results are summarized in Table 1.

Degenerating eggs collected at the same time as 2- and 8-cell eggs were devoid of enzyme activity.

Discussion

Using dehydroepiandrosterone as the substrate, Dickmann and his colleagues (Dickmann & Dey, 1973, 1974a, b; Dickmann & Gupta, 1974; Dey & Dickmann, 1974; Dickmann *et al.*, 1975a, b) demonstrated Δ^5-3β-HSD in tubal eggs of rats, hamsters and rabbits, and reported that the enzyme activity first appeared at the stage of morula–blastocyst transformation, and suggested that steroid hormones produced by the eggs at this stage controlled this transformation step. Dickmann & Dey (1974b) further examined the influence of (1) ligation of the uterotubal junction, (2) hypophysectomy, and (3) ovariectomy, and stated that Δ^5-3β-HSD activity in blastocysts was autonomous because none of these treatments had any effect.

In the present investigation, activities of Δ^5-3β-HSD and 20α- and 20β-HSD were found in hamster eggs at the 1- and 2-cell stage; the discrepancy with Dickmann's results may be due to the different incubation times or substrates used. Not only did the enzymes appear at the earliest tubal stages, but there was also no difference in activity between penetrated and unfertilized eggs, suggesting that fertilization has little influence on the development of the enzyme. The enzyme activities were still present at the blastocyst stage but had not risen at the time of blastocyst formation. These results therefore suggest that progestagens, shown to be progesterone, 20α- and 20β-hydroxypregn-4-en-3-one by the specificity of the substrates, in hamster eggs may participate, not only in blastocyst formation, but also in other physiological activities. It has been reported that rabbit blastocysts contain progesterone (Lutwak-Mann, 1971; Seamark & Lutwak-Mann, 1972) and 20α-hydroxypregn-4-en-3-one (Seamark & Lutwak-Mann, 1972), while pig blastocysts synthesize oestrogen as well as progesterone (Perry & Heap, 1973; Perry *et al.*, 1973).

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