Causes and consequences of early embryonic diversity in pigs

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Summary. Within 14 h of ovulation, follicular development in gilts was skewed towards a majority of mature follicles, based on their endocrine milieu. Oocyte maturation was also skewed, with a majority of the oocytes being meiotically more developed than the rest. Similarly, the pattern of ovulation in gilts was such that 70% of the follicles ovulated during a short period of time, while most of the remaining 30% ovulated over a more protracted period. This majority/minority pattern of both oocyte development and ovulation paralleled the distribution of development among 1-cell litter-mate embryos. Furthermore, oocytes of follicles predicted to ovulate first became the more developed embryos, while oocytes from later ovulating follicles became the lesser developed embryos. When these later ovulating follicles were destroyed by electrocautery, diversity in embryonic morphology was reduced by Day 12, and this reduction resulted from elimination of the lesser developed embryos. Genetic factors might also affect embryonic disparity, as SLA (swine leucocyte antigen complex) haplotype affected cleavage rates of embryos from miniature pigs. Results of various embryo transfer experiments demonstrated that the more developed embryos within a litter have a competitive advantage for survival over their less developed contemporaries. These lesser developed embryos, however, were just as viable as the more developed embryos after asynchronous transfer to recipients displaying onset of oestrus 1 day after the donors. The more developed embryos within the litter, by synthesizing more oestradiol than the smaller embryos, advanced uterine secretions. As a result, the lesser developed embryos probably became more susceptible to this new environment and eventually died in an asynchronous environment. Therefore, we suggest that early embryonic mortality directly relates to sequences of oocyte and follicular maturation, as oogenesis directs embryogenesis.

Keywords: pig; embryo; oocyte; follicle

Introduction

Diversity among litter-mate embryos is evident in swine from early cleavage (Perry & Rowlands, 1962; Hunter, 1972a) to somite formation (Anderson, 1978). Diversity among embryos also occurs naturally in other polytocous species (Gates, 1965; Hafez, 1962) and in monovulatory species, such as mares, after superovulatory procedures (Woods & Ginther, 1983). The relationship between diversity among embryos and subsequent mortality is complex. Estimates of the proportion of initially viable embryos that die during early gestation in pigs ranged from 25 to 35% (Hammond, 1921; Day *et al.*, 1959; Spies *et al.*, 1959; Perry & Rowlands, 1962). In comparison, Wright *et al.* (1982) observed that, within the morphological range of pig blastocysts recovered on Days 6–9, a distinct minority (20%) of embryos were smaller and contained less total protein. The extent to which these percentages are more than coincidentally related will be outlined in this review.

Causes of embryonic diversity

The causes of diversity among litter-mate embryos in pigs are not fully understood, but a list of potential factors might include differences in: follicular/oocyte maturation, ovulation, time of fertilization, sex-linked genes, SLA (swine leucocyte antigen complex) genotype, oviducal transport, hatching from the zona pellucida or uterine location. Based on observations in mice, variation among litter-mate embryos results from differences in time of fertilization (Gates, 1965), sex differences (Tsunoda et al., 1985; Seller & Perkins-Cole, 1987) and slow versus fast Ped (preimplantation embryonic development) genes (Verbanac & Warner, 1981; Goldbard & Warner, 1982). The following discussion will consider experimentation that either supports or negates the influence of each of these factors on embryonic diversity in pigs.

Follicular diversity within sows was first described by Foxcroft & Hunter (1985) and is updated by Hunter & Wiesak (1990). In the context of relating follicular diversity to embryonic diversity, Xie et al. (1990b) observed that follicular development, within gilts, was skewed towards a majority of follicles being more developed than a lesser developed minority. This conclusion was based on follicular fluid content of progesterone, oestradiol, androstenedione, testosterone and dermatan sulphate, and follicular wall content of prostaglandins F-2α and E-2 of gilts from 21 to 34 h after the onset of oestrus. The distribution of folliculr progesterone, oestradiol and prostaglandins F-2α and E-2 content were skewed among follicles within gilts. Likewise, the pattern of oocyte maturation within a gilt was skewed (Table 1; Xie et al., 1987). The percentage of further developed oocytes was 76 ± 4% (mean ± s.e.m.) and the remaining minority, 24 ± 4%. Collectively, follicular development and oocyte maturation contributed to early embryonic diversity, but these differences, according to the cytogenetic observations of Hunter (1974), were as small as 2–4 h.

Table 1. Number of oocytes from within a gilt (row) classified at various stages of meiosis*

Time (h) after onset of oestrus	Gilt No.	Stages of meiosis*				
		GV	GVBD	Met. I	Ana. I/Telo. I	Met. II
27	1	3	9			
27	2	4	1	15		
27	3			5	11	
27	4			1	1	9
27	5			3		16
27	6			4	2	8
30	7			2	2	12
30	8			1	2	9
30	9			1		13
30	10			1		16
30	11			2		11

*GV = germinal vesicle; GVBD = germinal vesicle breakdown; Met. I = metaphase I; Ana. I/Telo. I = anaphase I to telophase I; Met. II = metaphase II.

The duration and pattern of ovulation has, procedurally, been a difficult phenomenon to estimate. Burger (1952), Pitkjanen (1958) and Betteridge & Raeside (1962) concluded that ovulation in pigs occurred over a 1–3 h interval. The pattern of ovulation in gilts was further investigated by Pope et al. (1988), who observed 57 gilts at 34 h after the onset of oestrus and noted that ovaries of 18 of the gilts contained 6–8 mm follicles and no corpora haemorrhagica, 25 had corpora haemorrhagica along with follicles and 14 gilts had only corpora haemorrhagica (0, 5 to 95 and 100% completed ovulation, respectively; Fig. 1). Of the 25 gilts considered to be in the process of ovulating, one gilt had 1 corpus haemorrhagicum and 17 follicles (5% completed) and the 24 others had

10–17 corpora haemorrhagica and 1–4 follicles (68–95% completed ovulation, respectively). More ($P < 0.01$) gilts (24 of 25 *vs* 1 of 25) had between 68 and 95% than between 0 and 68% completed ovulations, respectively. Hunter (1972b) observed a similar pattern of ovulation in gilts at 44–46 h after injection with human chorionic gonadotrophin (hCG). The numbers of gilts with 0, 1–59, 60–94 and 100% completed ovulations were 3, 0, 15 and 23, respectively. The rarity of gilts observed with 0 and 70% completed ovulations suggested that a majority of follicles ovulated over a short period of time, while the remaining minority of follicles ovulated over a more protracted interval.
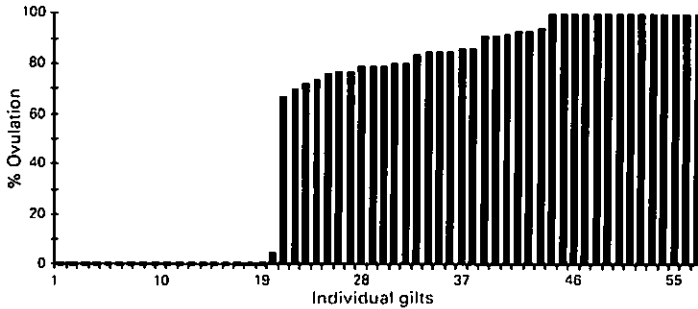

Fig. 1. Ranking of 57 gilts at 34 h after the onset of oestrus from 0 to 100% completion of ovulation (from Pope *et al.*, 1988).

Time to fertilization failed to influence diversity among embryos as it appeared to be constant in gilts mated shortly before ovulation, similar to the physiology of fertilization in mice (Bolton *et al.*, 1984). Xie *et al.* (1990a) flushed zygotes and one-cell embryos from the oviducts of mated gilts, 39–42 h after the onset of oestrus, and stained and classified them as described by Hunter (1974). The distribution of stained 1-cell embryos showed a skewed relationship with a majority being further developed and the minority being less well developed. In a number of gilts, for example, a minority of the oocytes were still in the second meiotic arrest, while the majority had resumed or finished the second meiosis. The complementary relationship between the distributions of oocyte maturation, ovulation and 1-cell development supported the observations of Hunter *et al.* (1987) that the number of pre-acrosome reacted spermatozoa within the oviducts was not 'rate-limiting' during fertilization. Likewise, it was concluded that oocytes of follicles that ovulated first became the more developed embryos and *vice versa* (Xie *et al.*, 1990a). This early disparity in follicular development extends beyond early post-fertilization events, as destruction of the later ovulating follicles by electrocautery eliminated the lesser developed embryos normally present by Day 12 (Day 0 = onset of oestrus; Pope *et al.*, 1988). These observations suggested that embryonic diversity throughout at least the first 12 days of gestation was predetermined by factors associated with oogenesis and follicular development.

Genetic factors might also influence embryonic diversity: Bazer *et al.* (1988) observed breed differences in that Chinese Meishan gilts experienced more uniformity of blastocyst development than did Large White gilts. Sex-linked genes have not been observed to be associated with morphological differences among embryos, but Ford *et al.* (1988) observed a relationship between variation in cleavage rates of embryos of miniature pigs and SLA haplotype. The content of DNA was determined in blastocysts flushed at Days 6, 9 or 11 from homozygous females (SLA^a, SLA^c, SLA^d) mated to homozygous males of the same genotype. Cleavage rates diverged between $SLA^{a/a}$ or $SLA^{c/c}$ and $SLA^{d/d}$ embryos between Days 9 and 11 (Fig. 2), demonstrating that genetic differences might influence embryonic disparity.

Oviducal transport, hatching from the zona pellucida and uterine location have no effect on embryonic diversity under physiological conditions. The rate of oviducal transport in pigs has been examined by several investigators (Assheton, 1898; Corner, 1921; Pomeroy, 1955; Oxenreider & Day, 1965), and it was concluded that embryos normally enter the uterus during a short interval
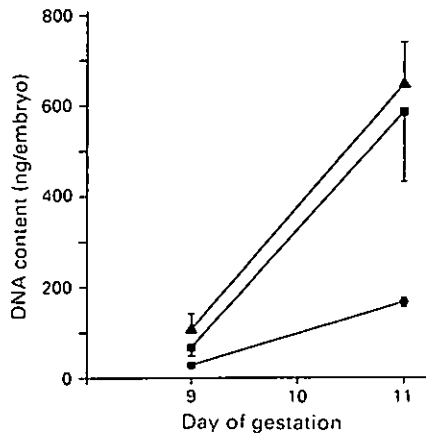
Fig. 2. Content of DNA from SLA$^{a/a}$ (▼), SLA$^{c/c}$ (■) and SLA$^{d/d}$ (●) matings on Days 9 and 11 of gestation. Values are mean ± s.e.m. for 8, 9 and 8 females (from Ford *et al.*, 1988).

(Alanko, 1965; Polge, 1966; Hunter, 1974; Hunter *et al.*, 1987). Perry & Rowlands (1962) and Broermann *et al.*, 1990 suggested that pig embryos enter the uterus independent of their stage of development after observing 5 and 13 gilts, respectively, in which embryos were entering the uterus at the time of necropsy. As 4- and 8-cell embryos were recovered from both oviducts and uteri of these gilts, and as no differences existed between the proportion of 4- or 8-cell embryos collected from either site, it was concluded that 8-cell embryos do not enter the uterus before 4-cell embryos. Likewise, Day-4 embryos transferred to the oviduct or uterus were not different morphologically on Day 12 (Broermann *et al.*, 1990). The time of hatching from the zona pellucida did not influence subsequent rates of embryonic development. When the zona pellucida of Day-6 blastocysts was removed enzymically and then the embryos were transferred, the Day-12 embryos that resulted were similar in morphological development to those from transferred zona-intact blastocysts (Table 2). Finally, restriction of pig embryos to a small portion of the uterus failed to affect embryonic development to the filamentous stage (Dziuk, 1968; Pope *et al.*, 1982a). Likewise, no relationship was observed between location within the uterus and morphology or protein content of recovered embryos (Anderson & Parker, 1976; Anderson, 1978).

Consequences of embryonic diversity

Consequences of diversity among litter-mate embryos are probably better understood than are causes. Differences in morphological disparity can now be related to subclasses of embryos that live or die during early gestation. Furthermore, the characteristic dissimilarities between these embryos allow us to propose some initial mechanisms whereby embryonic mortality occurs in pigs.

To determine whether embryonic mortality was a random event, Pope *et al.* (1982b) established pregnancy in gilts with two populations of genetically marked blastocysts, with one group 2 days older than the other (Days 7 *vs* 5, respectively) and each only 1 day out of synchrony with the recipients (Day 6). To assess that proportion of embryos surviving the transfer procedures compared with those surviving 'competition' during the first 25 days (Bazer *et al.*, 1969), half of the recipients were examined 5 days after transfer (Day 11) and the other half on Day 60. Conceptuses examined on Days 11 and 60 were identified by size and coat colour, respectively, to determine their original source (transferred Day-5 or -7 embryos). An additional group of Day-6 recipients received only Day-5 embryos and their survival rate was observed to Day 60. The results (Table 3) illustrated that Day-5 or -7 embryos could survive to Day 60, but when both groups of embryos

Table 2. Morphology of Day-12 blastocysts after transfer of zona-intact or zona-free embryos on Day 6 (from Broermann *et al.*, 1990)*

Zona	Recipient no.	Spherical 1–2 mm	3–5 mm	6–10 mm	Ovoidal	Tubular	Filamentous
Present	34	+			+	+	+ + + +
	90		+				+ + + + + +
	63		+ + +	+ + +	+ + + +	+	+
	11	+ +					+ + + + + + + +
	139				+ +	+ + + +	+
Not present	153	+					+ + + + + + + + +
	96		+ +			+	+ + + + + + +
	134				+ + +	+ + + + +	+ + +
	83	+ +					+ + + + + + + + + +
	36			+	+ +	+ + +	+ +

*Each '+' represents 1 embryo recovered on Day 12.

were transferred to the same uterus, the older, more developed, embryos had a preferential chance of survival. In a similar experiment, when Day-7 embryos were transferred to mated recipients on Day 6 or *vice versa* (Day-6 embryos to mated Day-7 recipients), Day-12 embryos of the former group of gilts were proportionally more advanced than embryos of the latter group (Pope *et al.*, 1986b). Allowing similarly treated gilts (transfer of Day-7 embryos to Day-6 recipients *vs* Day-6 embryos to Day-7 recipients) to progress to Day 30 resulted in more embryonic survival in the former recipients with proportionately more ovoidal, tubular and filamentous embryos than spherical embryos.

Table 3. Percentage survival of Day-5 and -7 pig embryos to Days 11 and 60 of gestation after transfer to Day-6 recipients (from Pope *et al.*, 1982b)

	No. of recipients utilized	No. of recipients pregnant	Day-5 embryos No. of embryos transferred	No. surviving	Survival/ recipient (%)	Day-7 embryos No. of embryos transferred	No. surviving	Survival/ recipient (%)
Day 11	10	8	45	19	$42\cdot3 \pm 10\cdot4^a$	42	18	$43\cdot1 \pm 12\cdot4^a$
Day 60	16	8	87	6	$8\cdot2 \pm 6\cdot9^b$	83	53	$62\cdot6 \pm 7\cdot7^c$
Day 60	10	5	88	35	$42\cdot0 \pm 9\cdot8$			

[a,b,c]Means with different superscripts within rows are different ($P < 0\cdot001$).

One might argue that the less developed embryos are smaller because they are inherently less viable and, therefore, are compensated for throughout early gestation (Fig. 3). Wilde *et al.* (1988) examined this phenomenon by segregating recovered Day-7 blastocysts into three groups; largest, intermediate and smallest. Four to 5 of the smallest and 4–5 of the largest were transferred to opposite, but ligated, uterine horns of synchronous (Day 7) or asynchronous (Day 6) recipients. By Day 12, the originally smaller blastocysts were less viable after synchronous transfer procedures than were the larger litter mates (Table 4). However, in the less advanced recipients, the smaller blastocysts survived as well as the larger blastocysts. These results support the concept that initially

most pig blastocysts are viable, and that the less developed blastocysts are more susceptible to an advanced uterine environment than are blastocysts that are morphologically more mature. Morgan *et al.* (1987a) have demonstrated that uterine advancement on Day 11 was embryocidal to lesser developed embryos.
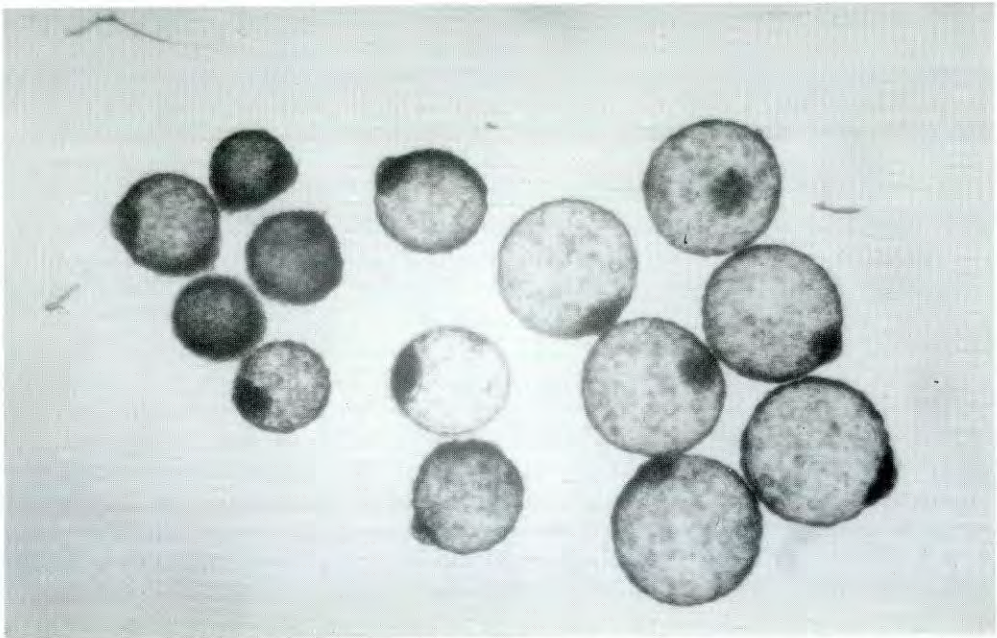

Fig. 3. Diversity among Day-7 litter-mate embryos (note the less, intermediate and more developed embryos from left to right).

Table 4. Embryonic survival (mean $\pm$ s.e.m.) after synchronous (Day-7 recipient) and asynchronous (Day-6 recipient) transfer of small and large Day-7 litter-mate blastocysts (from Wilde *et al.*, 1989)

Type of transfer	Embryonic survival (%)	
	Small Day-7 blastocysts	Large Day-7 blastocysts
Synchronous (N = 9)	38.3 ± 5.8^a	73.9 ± 5.8^b
Asynchronous (N = 7)	75.4 ± 6.6^b	70.7 ± 6.6^b

[a,b]Means with different superscripts differ ($P < 0.01$).

The mechanisms by which the lesser developed embryos become subjected to an advanced uterine environment might be unique to pigs. Pig blastocysts synthesize increasing amounts of oestradiol from Days 10 to 12 (Perry *et al.*, 1973, 1976; Heap *et al.*, 1977; Hoversland *et al.*, 1983; Fischer *et al.*, 1985; Stone *et al.*, 1986). The elegant experiments of Geisert *et al.* (1982a, b) demonstrated that exposure to oestradiol could advance uterine secretions. As the more developed embryos synthesized more oestradiol than did lesser developed litter mates (Ford *et al.*, 1982; Pope,

1988), segments of the gravid uterus might have become more advanced, biochemically, than others (Pope & First, 1985). Initially, as intrauterine migration ceased (Polge & Dziuk, 1970), these advanced microenvironments were localized in juxtaposition to the more developed blastocysts. The initial diversity of 2–4 h during oocyte maturation might have expanded by Days 10 to 12 as the more developed blastocysts took advantage of these more complex secretions and grew more rapidly. Ultimately, as embryonic synthesis of oestradiol increased, all portions of the uterus became exposed to oestradiol and asynchronous uterine environments developed adjacent to the lesser developed embryos (Pope, 1988). In support of the concept that by Day 12 all portions of the uterus were exposed to oestradiol, the embryotoxic effects of exogenous oestradiol were more apparent on Days 9 and 10 than 12 and 13 (Pope *et al.*, 1986a): exposure to oestradiol on Days 12 and 13 was coincident with increased synthesis of oestradiol by blastocysts. The specific mechanism(s) by which an advanced uterine environment caused the demise of the lesser developed embryos remains unknown. Gries *et al.* (1989) observed that the loss of specific polypeptides was associated with oestrogen-induced advancement of the uterus. Perhaps oestradiol from the more developed embryos alters endometrial secretion of some component(s) critical for survival of the lesser developed litter mates.
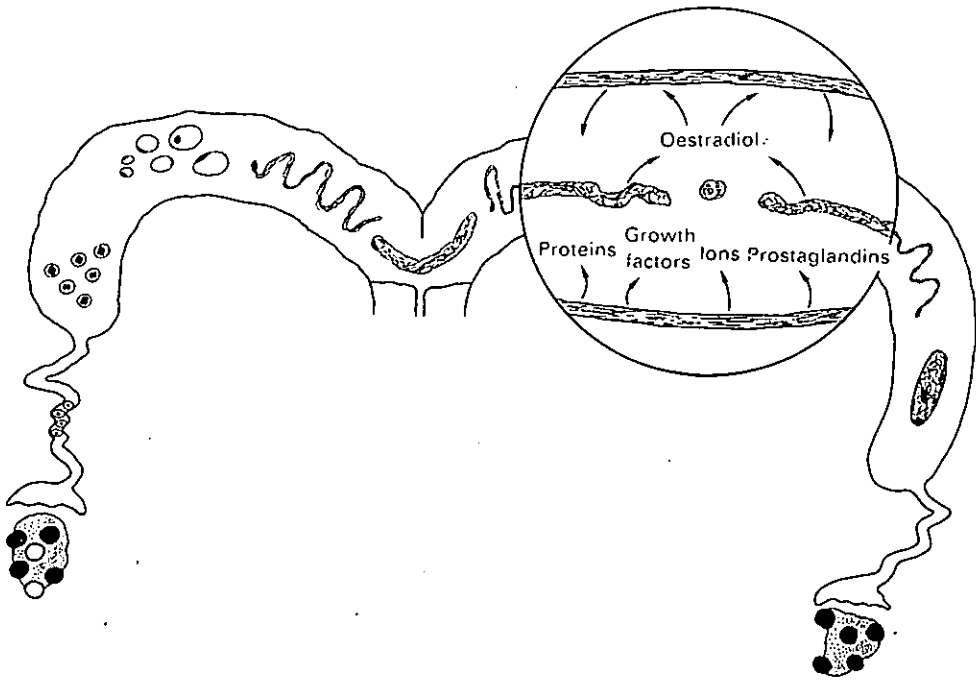

Fig. 4. A model illustrating the sequential events, from left to right, accounting for embryonic diversity. A majority of follicles ovulate before the protracted ovulation of the remaining minority of follicles. This initial disparity among embryos exists throughout blastulation and elongation. By Day 12, oestradiol from the more developed embryos advances uterine secretions, leaving the less developed embryos in a precariously asynchronous environment.

Model

Wilson (1925) proposed over 60 years ago that events at oogenesis direct embryogenesis. In pigs, disparity in follicular development leads to diversity in oocyte maturation. As a result, the pattern

of ovulation is skewed and a majority of mature follicles ovulate before the minority of later developing follicles (Fig. 4). Time to fertilization, which is constant in gilts mated shortly before ovulation, fails to alter this relationship and embryonic development continues with a majority of further developed embryos intermixed with a minority of lesser developed embryos. As the more developed embryos synthesize increasing amounts of oestradiol, uterine secretions are advanced in a complementary nature to the increasing complexity of blastocyst needs (Robl &·Davis, 1981). By Days 10–12, the lesser developed embryos are exposed to an asynchronous environment in which oestradiol from the filamentous embryos, for example, advances release of proteins (Geisert *et al.*, 1982a; Fazleabas *et al.*, 1983), growth factors (Simmen & Simmen, 1990), calcium (Geisert *et al.*, 1982a; Morgan *et al.*, 1987b) and prostaglandins (Zavy *et al.*, 1980) from the endometrium. Morgan *et al.* (1987b) and Gries *et al.* (1989) observed that the embryotoxic effects of uterine advancement were manifested 4–6 days later, as oestrogen administration on Days 9 and 10 caused the demise of embryos between Days 14 and 16. Curiously, the high survival rates of embryos of Chinese Meishan gilts might be associated with the unique characteristics of this breed, such as uniformity of blastocyst development (Bazer *et al.*, 1988) and shortened ovulation interval (F. Martinat-Botte, F. W. Bazer & M. Terqui, unpublished observations).

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