Follicular fluid electrolytes and osmolality in cyclic pigs

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Summary. Sodium and osmolal concentrations of porcine follicular fluid in the cyclic pig did not vary significantly during the oestrous cycle, and were similar to those in plasma. The K^+ concentration was greater in small (Days 12–13) and medium-sized (Day 16) follicles than in plasma or large (Day 18–oestrus) follicles of cyclic sows. In contrast, follicular fluid obtained from slaughterhouse material had higher potassium and osmolality, and lower sodium values, which are assumed to be due to post-mortem changes.

Introduction

A thorough understanding of the events which influence oocyte maturation requires examination of the chemical and physical properties of the follicular fluid microenvironment during the oestrous cycle. To date, most studies of oocyte physiology in the pig have utilized ovaries obtained from slaughterhouses. However, the possibility of post-mortem changes in these ovaries and the follicular fluid has been considered only occasionally, and may account for conflicting data on electrolytes and acid-base chemistries in porcine follicular fluid (Schuetz & Anisowicz, 1974; Chang, Jones, Ellefson & Ryan, 1976; Knudsen, Litkowski, Wilson, Guthrie, & Batta, 1978). For example, Knudsen *et al.* (1978) attributed variations in acid-base values in porcine follicular fluid from slaughterhouse material and that from known cyclic laboratory animals to differences between methods of sample collection and storage. Eiler & Nalbandov (1977) also found significant differences in follicular fluid steroid concentrations of such samples. Edwards (1974) suggested that follicular fluid for in-vitro studies should be collected from fresh samples to avoid degenerative changes.

The present report examines Na^+ , K^+ and osmolality of follicular fluid on different days of the oestrous cycle in the pig, and compares these values with similar determinations made from follicular fluid obtained from slaughterhouse material.

Materials and Methods

The cyclic sows used were sexually mature Yorkshire and Duroc sows (200–250 kg) with a history of at least one regular oestrous cycle. To obtain the maximum number of small (1–2 mm), medium (3–5 mm), and large (6–11 mm) follicles, sows were laparotomized on Days 12–13, 16 and 18–oestrus. Following anaesthesia (sodium pentobarbitone, 10 mg/kg), blood from the jugular vein was collected in heparinized tubes and immediately stored on ice until analysis. Fluid was collected with a 1-ml tuberculin syringe fitted with a 23-gauge needle from individual

follicles within 1–2 min of ovarian extirpation, and stored on ice. Fluid from follicles of similar size was pooled to provide an adequate volume (see below) for analysis. Plasma and follicular fluid were assayed on the day of the experiment, with the average time interval between collection and analysis being 2.5 h. For comparison, porcine ovaries were obtained from a slaughterhouse and placed in 0.9% (w/v) NaCl (unbuffered; pH 6.0) at room temperature. The time between slaughter and follicular fluid recovery was 3–4 h, with analysis on the same day. No distinction was made between atretic and non-atretic follicles for either group of samples.

Samples of blood and follicular fluid were centrifuged for 10 min at 7000 $g_{\max}$ to remove, respectively, blood cells and oocytes/granulosa cells. Aliquots (50 μ l) of the supernates were then assayed for Na^+ and K^+ using a flame emission photometer (IL243: Instrumentation Laboratories, Inc., Watertown, Massachusetts) with a precision of ± 1.0 mmol/l for Na^+ , and ± 0.05 mmol/l for K^+ . Additional aliquots (250 μ l) of the supernates were used for the determination of osmolality by freezing point depression on a 3D II osmometer (Advanced Instruments, Inc., Needham Heights, Massachusetts) with a precision of ± 2 mosmol/kg. Results (means $\pm$ s.e.m.) were tested for significance by Duncan's Multiple Range Test, and Student's t test where applicable (Steel & Torrie, 1960).

Results

The data presented in Tables 1 and 2 indicate that electrolyte and osmolal values in porcine follicular fluid varied with the method of sample collection. For example, potassium concentrations in follicular fluid of ovaries obtained from the slaughterhouse were 2- to 3-fold greater than in plasma or in fluid from follicles of similar sizes in cyclic pigs.

Sodium concentrations did not differ so greatly, but were lower in the slaughterhouse material.

The osmolality of ovarian fluid from cyclic pigs did not vary significantly with the stage of the oestrous cycle, and was similar to plasma values (Table 2). In contrast, the osmolality of ovarian follicular fluid in slaughterhouse materials was on average 9 mosmol/kg higher than that in plasma or follicular fluid of the cyclic pig, and varied inversely with the size of the follicle.

Table 1. Na^+ and K^+ concentrations in porcine venous blood and follicular fluid at different stages of the oestrous cycle

Group	Day of cycle	No. of sows	Follicle size (no.)	Na^+ (mmol/l)	K^+ (mmol/l)
I Plasma	—	5	—	140.0 ± 1.9 (5)	4.84 ± 0.21 (4)
II Follicular fluid from cyclic pigs	12–13	4	Small (155)	$145.2 \pm 0.6^*$ (4)	$5.55 \pm 0.27^\ddagger$ (6)
	16	4	Medium (81)	143.0 ± 1.3 (6)	$5.52 \pm 0.23^\ddagger$ (4)
	18–oestrus	4	Large (19)	$144.8 \pm 0.3^*$ (5)	4.92 ± 0.21 (5)
III Follicular fluid from slaughterhouse material	—	3	Small (200)	135.5 ± 1.3 (7)	$12.16 \pm 0.22^\ddagger$ (7)
	—	4	Medium (96)	140.7 ± 1.6 (10)	$10.27 \pm 0.53^\ddagger$ (10)
	—	4	Large (29)	138.9 ± 0.9 (8)	$8.89 \pm 0.28^\ddagger$ (8)

Values are mean $\pm$ s.e.m. for the no. of determinations given in parentheses.

* Value significantly greater than that for follicles of corresponding size in Group III ($P < 0.05$).

† Value significantly greater than those for plasma and follicles of corresponding size in Group II ($P < 0.01$).

‡ Value significantly greater than that for plasma ($P < 0.05$).

Table 2. Mean $\pm$ s.e.m. (no. of observations in parentheses) osmolal concentrations in porcine venous blood and follicular fluid

Group	Day of cycle	No. of sows	Follicle size (no.)	Osmolality (mosmol/kg)
I Plasma	—	5	—	300.7 $\pm$ 1.6 (5)
II Follicular fluid from cyclic pigs	12–13	3	Small (150)	302.6 $\pm$ 2.0 (5)
	16	3	Medium (43)	297.3 $\pm$ 1.5 (5)
	18–oestrus	6	Large (36)	305.1 $\pm$ 3.0 (8)
III Follicular fluid from slaughterhouse material	—	16	Small (332)	313.0 $\pm$ 0.8*† (8)
	—	10	Medium (197)	308.1 $\pm$ 0.9*† (20)
	—	6	Large (26)	308.4 $\pm$ 2.0† (10)

* Values significantly different from those in plasma and follicles of corresponding size in Group II ($P < 0.05$).

† Value significantly different from that in plasma ($P < 0.05$).

Discussion

The data reported here comprise the first examination of Na^+ , K^+ , and osmolal concentrations of follicular fluid obtained from cyclic pigs on known days of the oestrous cycle. The values of Na^+ and K^+ of porcine plasma corroborate the earlier findings of Cummings & Kaiser (1959), and are within the normal range for human plasma. Values of osmolality for porcine plasma are slightly higher than those of other species, such as man (Edwards, 1974) and cow (Olds & VanDemark, 1957).

In the cyclic pig, the K^+ concentration of porcine follicular fluid varied slightly during the days of the oestrous cycle examined. Though significant compared to plasma, it is not clear if the slightly higher K^+ values from small and medium-sized follicles reflect a physiological process of follicular maturation. Another possible explanation for this difference is extraneous contamination, since a larger number of small and medium-sized follicles had to be aspirated to provide sufficient volume for the analyses. Previous investigators (Schuetz & Anisowicz, 1974; Chang *et al.*, 1976) have reported even higher K^+ values in porcine follicular fluid from ovaries obtained from a slaughterhouse. The higher K^+ values of follicular fluid found in the slaughterhouse preparation suggest that the method of collection and storage of follicular fluid is critical. The anoxia and intrafollicular acidosis (Knudsen *et al.*, 1978) which result from ischaemia before collection of follicular fluid could lead to post-mortem degeneration of granulosa cells with resultant loss of intracellular K^+ into the follicular fluid. Concomitant with this shift in K^+ , Na^+ moves in the opposite direction. The inverse relation between follicle size and follicular fluid K^+ in the slaughterhouse material is also consistent with this reasoning, since smaller follicles would accumulate relatively more K^+ , due partly to the greater surface area:volume ratio.

The Na^+ concentration of porcine follicular fluid was relatively constant throughout the oestrous cycle. Schuetz & Anisowicz (1974) also reported that Na^+ was invariant with respect to follicular size, although the mean value for Na^+ reported by these investigators was lower than any of the values found in the present study. In the slaughterhouse material the Na^+ values for follicular fluid were lower than those obtained from fresh follicles. Chang *et al.* (1976) noted a similar pattern between "ice-stored" and "fresh" preparations, the latter more closely approximating serum Na^+ . Lower Na^+ values in follicular fluid of the slaughterhouse preparation may

again reflect partial equilibration of Na^+ and K^+ between granulosa cells and follicular fluid, as suggested above. In addition, it is possible that Na^+ and K^+ within the follicle may equilibrate with the medium in which the ovaries are kept when transported to the laboratory. At the end of ovarian storage, this medium (0.9% NaCl) was found to have a K^+ value of 10.05 ± 0.45 mmol/l, which we attribute mainly to haemolysis of red blood cells resulting from ovarian excision.

The present observations are particularly significant in light of the investigations of Powers & Biggers (1976), who demonstrated that removal of external Na^+ from a chemically defined medium, or increasing the K^+ permeability of the oocyte, affected the maturation (germinal vesicle breakdown) of the isolated mouse oocyte. The hyperosmolality of porcine follicular fluid from ovaries obtained from a slaughterhouse is also of interest in view of the finding of McGaughey (1977) that osmolalities of this magnitude can cause poor oocyte maturation in in-vitro cultures.

Our findings suggest that studies which utilize follicular fluid require critical attention to the limitations inherent in the use of slaughterhouse preparations.

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