The presence of enzymes, namely, acid phosphatase, alkaline phosphatase and amylase in buck semen were studied in the present investigation. Acid phosphatase activity was found to be insignificant. Alkaline phosphatase activity varied from 30·00 to 247·50 K.A.U. with a mean of 107·14 ± 31·84 K.A.U. Marked amylase action was detected with a mean unitage of 116·10 ± 7·58 Somogyi units.

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OBSERVATIONS ON OVARIAN ASCORBIC ACID AND CHOLESTEROL DURING INDUCED OVULATION IN THE SKIPPER FROG *RANA CYANOPHLYCTIS* SCHNEIDER

THE physiological basis of ovulation in Amphibis is complicated due to the fact that the release of gravid eggs from ovaries results from an integrated functioning of the different endocrine organs, such as adrenals[1] and thyroids[2] in addition to the pituitary[3]. However, the pituitary homogenates induce ovulation better than steroids while the latter are more efficient inducers of the oocyte maturation[4]. It may be pointed out that in all these studies the response of ovaries to ovulation induced by a variety of agents has been evaluated on a common morphoogical basis, *ie.*, by counting the number and rate of eggs released *in vivo* or *in vitro*. It is therefore essential to have information on the alterations occurring in ovary, particularly at biochemical levels, during ovulation induced by different agents at any given time and at different periods of the annual cycle and hence this investigation was undertaken.

Adult female frogs, *R. cyanaphlyctis*, weighing 30–45 g were collected during the months of June and October[5]. Ovulation was induced by administering pituitary and hormones intraperitioneally. The hormones and the doses employed[6] were methyl testosterone, progesterone and DOCA (Sigma Chemicals and Co.). They were dissolved in olive oil (0·2 ml oil contained the dose, Table I). Eltroxine tablets (Glaxo) 0·1 mg were powdered and suspended in distilled water. One ml of this was administered in combination with a single pituitary. All frogs were maintained individually in aerated aquaria under laboratory conditions (26 ± 1° C). Frogs from the breeding period (Table I) were autopsied exactly at the 8th hour after the treatment[7-8] while those

TABLE I

Response of ovary to ovulation induced by pituitaries and hormones

	Treatment		per 100 mg weight of ovary (M ± S.E.)		Response*
			Ascorbic acid (μg)	cholesterol	
1	Control dist. H_2O 1 ml	(5)	20·62±0·5	1·93±0·14	–
2	Control olive oil	(5)	19·72±1·11	1·72±0·03	–
3	Treated 1 pituitary	(5)	22·83±1·10	1·61±0·09	–
4	Treated 2 pituitaries	(5)	22·96±1·51	1·35±0·04	++
5	Treated 4 pituitaries	(5)	22·43±1·32	1·35±0·01	+++
6	Treated 1 mg progesterone	(5)	26·15±4·13	1·81±0·26	+++
7	Treated 1 mg DOCA	(5)	19·88±0·86	1·37±0·08	+++
8	Treated 2 mg methyl testosterone	(5)	21·50±5·29	2·04±0·02	+
9	Treated 0·1 mg eletroxine + 1 pituitary	(5)	24·16±3·27	1·76±0·30	++

* Response + = 1 to 10 eggs released
++ = 10 to 50 eggs released
+++ = 50 or more eggs released
Number in parenthesis is the number used.
M ± S.E. = mean in relation to standard error.

Cholesterol	
1 *vs.* 3 *t* = 2·14	$P < 0·05$
1 *vs.* 4 *t* = 3·40	$P > 0·001$
1 *vs.* 5 *t* = 3·44	$P > 0·001$
1 *vs.* 7 *t* = 3·54	$P < 0·001$

TABLE II

Temporal response of ovary during pituitary stimulation in October

Treatment		per 100 mg weight of ovary (M ± S.E.)		Response*
		Ascorbic acid (μg)	cholesterol (mg)	
0 Non-gravid	(11)	47·43±2·64	1·26±0·23	—
1 control dist. H_2O	(10)	65·52±6·75	1·59±0·18	—
2 Treated 2 hours	(9)	38·35±2·60	1·26±0·04	—
3 Treated 4 hours	(9)	31·57±2·60	1·06±0·05	+
4 Treated 8 hours	(8)	26·50±1·24	1·09±0·39	+++
5 Treated 12 hours	(8)	20·10±1·86	1·02±0·03	+++
6 Treated 16 hours	(12)	48·72±2·78	0·98±0·07	+++

* Response as in Table 1.

Number in parenthesis is the number used. M ± S.E.—mean in relation to standard error.

		Ascorbic acid		Cholesterol
1 *vs.* 2	$t = 4·02$	$P < 0·001$	$t = 2·24$	$P < 0·05$
1 *vs.* 3	$t = 4·82$	$P < 0·001$	$t = 2·86$	$P > 0·01$
1 *vs.* 4	$t = 5·86$	$P < 0·001$	$t = 3·27$	$P > 0·001$
1 *vs.* 5	$t = 6·29$	$P < 0·001$	$t = 4·94$	$P < 0·001$
1 *vs.* 6	$t = 2·38$	$P < 0·05$	$t = 3·67$	$P > 0·001$

from the post-breeding period (Table II) were autopsied at intervals. Ovulatory response was graded and ovaries were removed rapidly after pithing, weighed on a torsion balance and transferred to a cold moist chamber. Ascorbic acid and cholesterol were estifated following the prodedures of Das Gupta *et al.*,[9] and Bloor Sackett (modified) as described by Koche and Hanke[10] respectively. The readings were taken from Klett-Summerson photoelectric colorimeter.

It may be observed (Table I) that ascorbic acid content of ovary remains more or less unchanged in response to treatment with pituitaries and hormones during the breeding season while cholesterol records depletions depending on the agent employed. Pituitary homogenates and DOCA decrease cholesterol but progesterone, methyl-testosterone and eltroxine have no effect. A scrutiny of the post-ovulatory ovary shows that it has released all gravid eggs by about 8 hours after treatment when optimum concentrations of pituitaries, progesterone and DOCA are used. On the other hand ovary during the post-breeding season (Table II) contains higher amounts of ascorbic acid which depletes rapidly upon pituitary stimulation. The peak period of ovulation and the maximum decrease in ascorbic acid correspond with each other. Similar levels are noticed during the breeding season. A slight decrease in cholesterol may be noticed in the control ovaries from the breeding to the post-breeding season but its response to treatment with pituitary remains the same.

Seasonal variations are reported to occur in the ascorbic acid and cholesterol content of ovaries in this species[11] and ovaries and adrenals in *Rana hexadactyla*[12]. They have been associated with alterations in the gonadotrophin activity during the annual dycle[7,8,12]. The present investigation shows that ascorbic acid content of ovary is low during the breeding season and on pianitary stimulation no change occures in it. Ovary during the post-breeding season contains higher levels which decrease rapidly during pituitary stimulation. This contrasting response may be due to the fact that ovary is under the stimulation of the endogenously sedreted gonadotrophins, particularly LH, from the pituitary during June and that the synthesis and/or the release of this factor decreases towards October[8]. Both pituitary homogenates and DOCA decrease ovarian cholesterol while progrestone, methyl testosterone and Eltroxine have no effect. This lends support to the belief that the exogenously administered hormones may have an action indirectly through the mediation of the pituitary of the recipient animal in addition to their direct one[6]. Thus, response of ovaries

during ovulation induced by different agents depends not only on the physiological status of pituitary, adrenals and thyroids[1–3] but also on ovaries and the period at which they are used since precise variations in ascorbic acid and cholesterol contents occur during the annual cycle.

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AWARD OF RESEARCH DEGREES

Karnatak University, Dharwad, has awarded the Ph.D. degree in Physics to Sri M. R. Gorbal.

Kakatiya University, Warangal, has awarded the Ph.D. degree in Mechanical Engineering to Sri B. G. Krishna Reddy; Ph.D. degree in Chemistry to Sri K. Krishna Pillai.