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Timing of fertilization in mammals: Sperm cholesterol/phospholipid ratio as a determinant of the capacitation interval

(interspecies correlations/sperm cholesterol efflux/acrosome reaction)

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ABSTRACT **A survey of species differences in the duration of capacitation, T, has revealed that they closely correlate with sperm cholesterol/phospholipid mole ratios, $R : T = 8R - 1$ ($r^2 = 0.97$, in which r is Pearson's correlation coefficient). Because uterine cells displayed low relative cholesterol concentrations, spermatozoa evidently experience a negative external cholesterol gradient (positive phospholipid gradient) during capacitation. A decrease in sperm R-value is suggested, therefore, to accompany capacitation. The idea received strong support from a kinetic analysis of capacitation intervals, based on the rate of cholesterol efflux from sperm cells *in utero*. Lipid-binding serum proteins in uterine fluid are attributed with removing a sterol barrier to the Ca^{2+}-facilitated membrane fusion that initiates the acrosome reaction. Tight cell junctions prevent permeation of the male generative tract by these proteins (capacitation factors). Furthermore, seminal plasma contains a decapacitation factor, identified as a membrane vesicle (cholesterol donor) component of this fluid, that reverses capacitation. Initiation of the sperm acrosome reaction among mammals could be the first fusion process found to be physiologically modulated through the membrane bilayer cholesterol level.**

Cells frequently display an intriguing sense of time. This is certainly conspicuous during embryogenesis of a complex organism. Even before fertilization, however, mammalian gametes change with time. Within hours of ovulation, eggs can age and become nonfertilizable. By contrast, spermatozoa acquire fertilizing capacity only after an interval in the uterus or oviduct (1). Mating normally precedes fertilization by several hours in these species for this reason. Molecular clocks of some kind appear to be set in motion as each gamete commences its passage to the site of fertilization.

The sperm's "clock" can be reset. This fact was discovered after exposing uterine-capacitated rabbit sperm to seminal plasma (2). Decapacitated spermatozoa regain fertilizing ability in the uterus, and recapacitation occurs at approximately the original rate (3). The transformation of sperm to a capacitated state is a unique feature of mammalian fertilization. It facilitates an intraspermatozoan membrane fusion initiating the acrosome reaction. By contrast with capacitation, the acrosome reaction is nearly universal (some teleosts lack it) during fertilization of animal eggs. Among mammals, exposure of hydrolytic acrosomal enzymes assists sperm penetration through the vitelline membrane and outer cellular and mucoprotein coats of the ovum.

Although the molecular events responsible for this membrane fusion are presently unclear, there is increasing evidence that capacitation influences sperm membrane lipids. We have proposed that capacitation involves a reversible lowering of the cholesterol (Chol)/phospholipid (PL) ratio in sperm membranes (4). Removal of the sterol destabilizes the plasma membrane, it is theorized, promoting its fusion with an underlying membrane, which forms the upper surface of the acrosome vesicle located in the anterior dorsal part of the sperm head. Membrane lipids are heterogeneous; however, several membrane attributes, including fusion potential, respond to changes in Chol level under experimental conditions.

Investigations spanning 30 yr reveal large species differences in the duration of capacitation. The cause of this variability is unknown. This report gives a survey of available data aimed at evaluating a hypothesis that these temporal variations arise from differences in the amount of sperm Chol. In particular, the kinetics of Chol efflux from sperm cells have been examined to explain the results. The action of decapacitation factor (DF) and generative tract/blood barriers are also considered in terms of the proposed mechanism. Evidence is presented that strongly indicates sperm capacitation removes a membrane/sterol barrier to fertilization, under physiological conditions.*

Capacitation interval and sperm cholesterol

Capacitation intervals *in vivo*, corresponding to half-maximum fertilization (sperm penetration) rates, are available for porcine (6), rat (7), rabbit (8), and ovine (9) sperm cells (Table 1). Bull sperm cells fertilize follicular oocytes, when preincubated at least 3 hr within a uterus or oviduct excised from an estrous cow (10). Human spermatozoa were capacitated by incubation in Bavister's medium, with residual follicular fluid, after 7 hr (11). Gamete transport rates to the site of fertilization have been disregarded.

Among these six species, capacitation intervals (1.5–7 hr) correlate closely with sperm Chol/PL ratios (0.35–0.99). Relevant lipid data (Table 1) were obtained from the results of Darin-Bennett and White (12) for ram, bull, rabbit, and human and from Komarek *et al.* (13) and Davis (4), respectively, for the boar and rat. The amount of Chol in boar sperm was deduced from their Chol/PL ratio (13) and quantitative PL data (14). On the average, spermatozoa from these species contain $18.3 \pm 0.79 \times 10^8$ Chol and PL molecules per sperm and nearly 1.7 PL molecules per Chol molecule. Free Chol, which might include desmosterol, and PL comprise around 80% of sperm lipids (15). In view of their quantitative significance, the physiological importance of these lipids for sperm fertilizing capacity (Table 1) and cold shock susceptibility (12) seems plausible.

Phospholipid fatty acyl chain varieties in spermatozoa from five of the species (16) and fatty acids of rat sperm (17) also correlate with capacitation interval (Table 1). Thus, mole fractions of saturated chains exceeding myristate in length, X_s [number

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Abbreviations: Chol, cholesterol (sterol alcohol); DF, decapacitation factor; PL, phospholipid.

* Ref. 5 is a preliminary report of these results.

Table 1. Capacitation interval and lipid composition of spermatozoa from various mammals*

Source	T, hr	Molecules × 10^8/sperm cell			PL fatty acids			
		Chol	PL	Chol + PL	X_s†	Mean C-chain length	R	$X_{Chol} + X_s$‡
Ram	1½ (9)	4.33	11.56	15.89 (12)	0.24	19.8 (16)	0.37	0.36
Boar	2 (6)	5.59	15.83	21.42 (13, 14)	0.23	20.2 (16)	0.35	0.34
Bull	3 (10)	5.36	11.99	17.35 (12)	0.23	20.0 (16)	0.45	0.37
Rat	3¾ (7)	7.10	12.26	19.36 (4)	0.45§	17.5§ (17)	0.58	0.58
Rabbit	6 (8)	8.47	9.67	18.14 (12)	0.46	18.8 (16)	0.88	0.65
Man	7 (11)	8.63	8.71	17.34 (12)	0.49	19.0 (16)	0.99	0.64

* Numbers in parentheses are references. T, capacitation interval; R, Chol/PL ratio; X_{Chol}, mole fraction of Chol; X_s, mole fraction of saturated fatty acids in total PL fatty acids.
† Data exclude myristate.
‡ $X_{Chol} + X_s = (R + 2X_s)/(R + 2)$.
§ Sperm cell-derived free fatty acids.

of saturated fatty acid chains (C > 14)/ total number of fatty acid chains], yield the following relationship with capacitation interval (T) on least squares regression analysis: $T = 15\,X_s - 1.4$, $r^2 = 0.78$, in which r is Pearson's correlation coefficient. In contrast, their carbon chain lengths (19.2 ± 0.05) show little species variation. The Chol plus saturated fatty acid mole fraction, X_{Chol+s} (number of Chol molecules plus saturated fatty acid chains/number of Chol molecules plus the total number of fatty acid chains), also correlates with capacitation interval: $T = 14\,X_{Chol+s} - 3$ ($r^2 = 0.86$). However, sperm Chol/PL ratios, R, provide a superior correlation: $T = 8R - 1$ ($r^2 = 0.97$; 0.86–0.998; 95% confidence interval).

This correlation demonstrates uniquely that Chol levels, relative to PL, represent a physiologically significant molecular determinant of capacitation. The apparent, albeit weaker, effect of saturated PL fatty acyl chains on the capacitation interval apparently results from a positive correlation between their level and Chol concentration in mammalian sperm cells.

Membrane/sterol barrier to fertilization

The foregoing result provides compelling evidence that Chol–PL interactions in sperm membranes influence capacitation. Support for membrane involvement can be found also in the data given by Table 1.

Comparing Chol mole fraction, X_{Chol} (molecules of Chol/Chol plus PL molecules), and PL mole fractions, X_{PL} (molecules of PL normalized by the species average of Chol plus PL molecules), reveals (see Table 1) that sperm Chol and PL levels are inversely correlated: $X_{Chol} = 0.77 - 0.64\,X_{PL}$ ($r^2 = 0.74$). The interchangeability of both amphipathic molecules in a membrane PL bilayer provides a basis for this correlation. In view of our previous finding, this result clearly supports the idea of a membrane/sterol barrier to fertilization.

As in other eukaryotic cells, high Chol concentrations characterize plasma membranes from rat spermatozoa (17). From the distribution of the polyene antibiotic, filipin, it is evident that Chol molecules occur in both inner and outer leaflets of this membrane and, to a lesser extent, in the Golgi-derived acrosomal membrane (18). Nuclear and mitochondrial membranes characteristically possess low Chol levels.

Evidence is presented in the next three sections that favors Chol depletion through the plasma membrane as the molecular mechanism responsible for the capacitation transformation.

Table 2. Chol/PL mole ratios in uterine cells, spermatozoa, and seminal plasma from various mammals

Lipid source	No. of species	Chol/PL,* $\bar{x}$ ± SEM
Uterine cells		
Estrogen-dominated	3	0.163 ± 0.051 (19–21)
Progesterone-dominated	3	0.260 ± 0.055 (19–21)
Spermatozoa	8	0.529 ± 0.097† (4, 12–14, 25)
Seminal plasma	7	0.548 ± 0.188† (12, 13, 17, 25–29)

* Means derived from between 5 and 10 determinations. Numbers in parentheses are references.
† Significantly greater than uterine cells ($P = 0.003$; t-test).

Sperm–uterus Chol gradient

Consistent with passive efflux of exchangeable sperm Chol during capacitation, the uterus provides a negative external sterol gradient (positive PL gradient). The mean Chol/PL ratios found for sperm (0.52) and seminal plasma (0.55) exceed those of estrogen-dominated (0.16) and progesterone-dominated (0.26) uterine cells (Table 2). These uterine data are from studies on the mouse (19), rat (20), and sow (21). Okey *et al.* (21) assayed for uterine cell lecithin, which is assumed to comprise half of the total PL in porcine generative tract tissue (22). Higher uterine fluid lipid levels follow administration of progesterone (23), and the steroid can be seen to elevate the uterine Chol/PL ratio (Table 2). This increase approaches statistical significance ($P = 0.141$). An increase in the ratio could help suppress uterine capacitation ability among progesterone-treated rabbits (24).

Table 2 includes results for spermatozoa from buffalo (25) and stallions (26) in addition to the previous species (Table 1). Seminal plasma data are from the following sources: boar (13), bovine (25, 27), buffalo (25), human (12, 28),† rabbit (17), ram (29), and stallion (26). Ejaculated spermatozoa and seminal plasma have comparable Chol levels (Table 2): R(seminal plasma) = 1.52 R(sperm) − 0.25 ($r^2 = 0.90$). Therefore, uterine-capacitated sperm should experience a positive sterol gradient if returned to this fluid. A reversible loss of fertilizing ability is known to follow exposure to seminal plasma (3).

Chol binding by uterine fluid

As is well known, Chol can be readily depleted from eukaryotic cells. Sterol transfer rates are low, however, in the absence of serum or albumin (30). Consequently, serum-derived sterol acceptors may be anticipated in uterine fluid. Moreover, their concentration and ligand affinity presumably parallel changes in uterine capacitation ability. Results supporting both propositions have recently been obtained in this laboratory.

Uterine fluid adsorption of sperm Chol was demonstrated

† Data of Poulos and White (28) indicate there are 11.88 × 10^8 PL molecules per human sperm, and this exceeds a more recent estimate reported from this laboratory (12). The latter has been chosen because it includes both Chol and PL determinations.

through gel filtration chromatography on Sephadex G-100, with fluid obtained 1.5 hr after intrauterine insemination of rabbit spermatozoa bearing radioactively labeled Chol. Consistent with hormonal effects on capacitation (24), uterine fluid from females given chorionic gonadotropin (to induce ovulation) bound 3-fold more Chol than did fluid of does treated with progesterone (to inhibit ovulation). The distribution of radioactive Chol in fractionated uterine fluid suggested that globulins and albumin bind the sterol. Alterations in the concentration and sterol affinity of these proteins could explain hormone-induced changes in uterine capacitation ability. The distribution of these Chol-binding proteins in uterine fluid broadly agreed with the capacitation activity profile observed after elution of serum from a column of Sephadex G-150 (31).

Kinetics

The kinetics of Chol depletion provide a reasonable, quantitative model for capacitation.

Preovulatory uterine fluid can be regarded as an essentially 10-fold diluted serum fraction, devoid of high molecular mass components (32). Sieving by the uterus virtually excludes serum macromolecules exceeding 10^5 daltons. Lipoprotein elimination should cause a loss of Chol buffering capacity and facilitate the net depletion of cellular Chol (30). From experimental results obtained by Fogelman *et al.* (30), lipoprotein-deficient serum ($\rho \geq 1.21$) elutes Chol from leukocytes (mainly from the monocyte fraction) as efficiently as does lipid-depleted serum (solvent extracted), which displays a pseudo-first-order rate constant of about 1.2 hr^{-1} at a concentration of 36 mg of protein per ml. The resulting decrease in sperm Chol/PL ratio should relate to capacitation interval as

$$T = k^{-1} \ln\{[R(\text{sperm}) - R(\text{uterus})]/[R(\text{capacitation}) - R(\text{uterus})]\}. \quad [1]$$

Alternatively,

$$T = a \ln[R(\text{sperm}) - R(\text{uterus})] + b. \quad [2]$$

R(sperm) refers to the initial ratio (Table 1), and we may take R(uterus) to be 0.16 (Table 2). A least-squares analysis yields $a = 3.48$ and $b = 7.26$ ($r^2 = 0.97$). From these coefficients, $k = 0.29$ hr^{-1} and R(capacitation) = 0.28. Fig. 1 indicates the calculated and observed T values as a function of R(sperm).

This rate constant is of the same order of magnitude as would be expected from the data of Fogelman *et al.* (30), when the protein concentration is 8 mg/ml ($1.2 \times 8/36 = 0.27$ hr^{-1}), which approximates the protein concentration of uterine fluid (32). The magnitude of these rate constants is also consistent with the observed kinetics of [^{14}C]Chol efflux from rabbit sperm *in utero* (unpublished data).

The critical Chol/PL ratios for capacitation appear to be of the order of 0.28. In contrast with R(sperm), R(capacitation) has been assumed to be constant between species. As they are whole cell ratios, higher ratios probably occur in capacitated sperm plasma membrane (17). However, the kinetics of Chol depletion, in relation to capacitation, would remain unchanged (Eqs. 1 and 2) should Chol/PL ratios in sperm and uterine plasma membrane exceed their respective whole cell ratios by a similar margin. Present evidence does not preclude PL transfer, as well as Chol depletion, during capacitation. At a minimum, this would change the explicit form of the rate constant, k.

A notable feature of the proposed model is the low Chol/PL ratio associated with capacitation. Significantly, the Chol/PL ratio is elevated among progesterone-dominated uterine cells (Table 2). Although sperm cells fail to capacitate in the uterus of a progesterone-treated female (24), possibly because R(uterus) is > R(capacitation), the model allows partial capacitation in these circumstances. Bedford (33) reported the predicted shortening of capacitation, after maintaining rabbit spermatozoa for 14–20 hr in the uterus of a pseudopregnant doe. He concluded, "capacitation is not an all-or-none phenomenon for any one sperm." Present findings indicate capacitation is progressive and reversible.

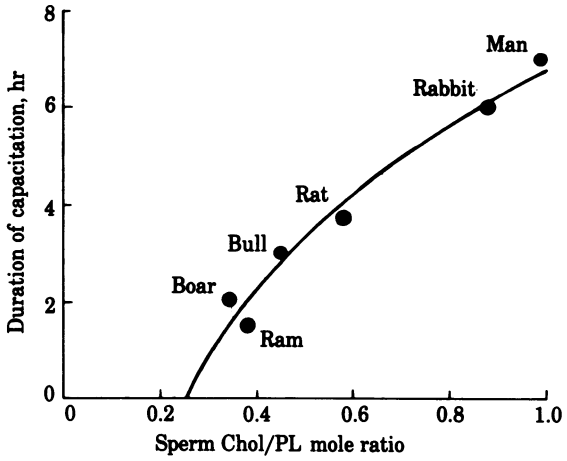

FIG. 1. Relationship between the duration of capacitation and sperm Chol/PL ratio. ●, Observation with indicated species; ——, estimate derived from Eq. 1. Correlation coefficient: $r^2 = 0.97$.

DF

An acceptable model for capacitation should suggest how the transformation is reversed by an inhibitor in seminal plasma. Because restoration of Chol depleted during sperm capacitation could conceivably explain decapacitation, the model described satisfies this requirement. In fact, the proposed mechanism receives strong support from studies on the identity and mode of action of DF.

Several lines of evidence indicate DF is a Chol-bearing membrane vesicle component of seminal plasma, and an elevation of the sperm Chol/PL ratio has been associated with decapacitation (4). Moreover, a DF-like action has been observed in synthetic PL vesicles containing Chol (34).

Some uncertainty about the identity of DF may be traced to an early speculation by Bedford and Chang (35), who implicated a mucoprotein. At the time it was not realized that DF can endure extensive proteolysis, rendering unlikely a protein inhibitor. They were led to this view because addition of cold ethanol precipitated DF from rabbit seminal plasma. However, rabbit seminal plasma DF vesicles, which possess a glycocalyx, also precipitate under identical conditions (unpublished data). The glycoproteins that have been identified as DF actually underestimate its hydrodynamic molecular weight and molar activity (4). Hydrolysis of glycoproteins on the vesicle surface diminishes vesicle affinity for spermatozoa, and this is observed to retard the rate but not the extent of decapacitation (36).

An early investigation indicating that DF contains lipid (37) seems to have been discontinued after Pinsker and an associate (38) showed DF from the seminal plasma of bulls, but not rabbits, could be "extracted" from a sediment by water. The solubilized DF was no longer rapidly sedimenting. However, Pinsker's procedure probably changes these vesicles through freezing and thawing, osmotic shock, and resuspension after sedimentation. After sedimentation in an analytical ultracentrifuge, the vesicles from bull seminal plasma appear to form

small or low-density entities, which move only slowly in a centrifugal field (unpublished data). As DF exhibits species cross-reactivity, fundamental differences in the mechanism of decapacitation are implausible. Likewise, capacitation lacks species specificity (39). These facts offer indirect corroboration for a nonspecific lipid exchange mechanism.

It may be estimated that a decrease in the Chol/PL ratio of rabbit spermatozoa during capacitation from 0.88 to 0.28 (Eq. 1) would remove about 6×10^6 Chol molecules per sperm. As a DF vesicle from rabbit seminal plasma contains roughly 1.6×10^4 Chol molecules, decapacitation should require not less than 360 vesicles per sperm. Around 10^7 vesicles per sperm are used to produce decapacitation—a concentration well in excess of this minimum multiplicity.

Generative tract/blood barrier

Apart from DF, Ca^{2+} chelators and various enzyme antagonists, which include desmosteryl sulfate at low concentration in hamster spermatozoa, reduce the risk of premature acrosome reaction and autolysis. Additionally, both male and female generative tract/blood barrier apparently help orchestrate spermiogenesis, sperm retention within the male, capacitation *in utero*, and fertilization (Fig. 2). Tight cell junctions around the seminiferous tubules, excurrent ducts, and accessory glands minimize permeation of this barrier by albumin and other serum proteins, which could act as capacitation factors. Impairment of this barrier elevates their concentration in seminal plasma and fertility declines (40).

In contrast, the uterus is semipermeable to serum proteins, and, as previously noted, hormonally induced changes in its permeability parallel alterations in capacitation potential. Among women at midcycle, serum PL and total Chol (alcohol and ester) concentrations decrease by 12% and 20%, respectively (41). Cervical mucus lipids, however, decrease by two-thirds, and the Chol/PL ratio declines from 1.02 to 0.41 (42), which should allow at least partial capacitation of human sperm at this site. Uterine cell Chol/PL ratio is also lowered by estrogen (Table 2). With transuterine permeation, sterol-binding serum proteins must equilibrate to the lower relative Chol level, enhancing their acceptor activity for capacitation.

As depicted in Fig. 2, the cervix excludes seminal plasma from the uterus and regulates inflow of suitable numbers of motile sperm cells among most mammals, including man (39). Within the uterus, ejaculated sperm cells rapidly divest adhering seminal plasma proteins. While decoating is an initial step, it does not represent capacitation (4). Protein- and Chol-free areas have been found in freeze-fracture replicas of plasma membrane and outer acrosomal membrane from capacitated sperm (18). Fusion at these sites is believed to initiate the acrosome reaction.

Initiation of the acrosome reaction

Induction of the acrosome reaction may be the *raison d'etre* for capacitation, as it seems to be the sole impediment to fertilization by noncapacitated spermatozoa (4). Recent evidence discounts the relevance of variations in sperm motility (43, 44).

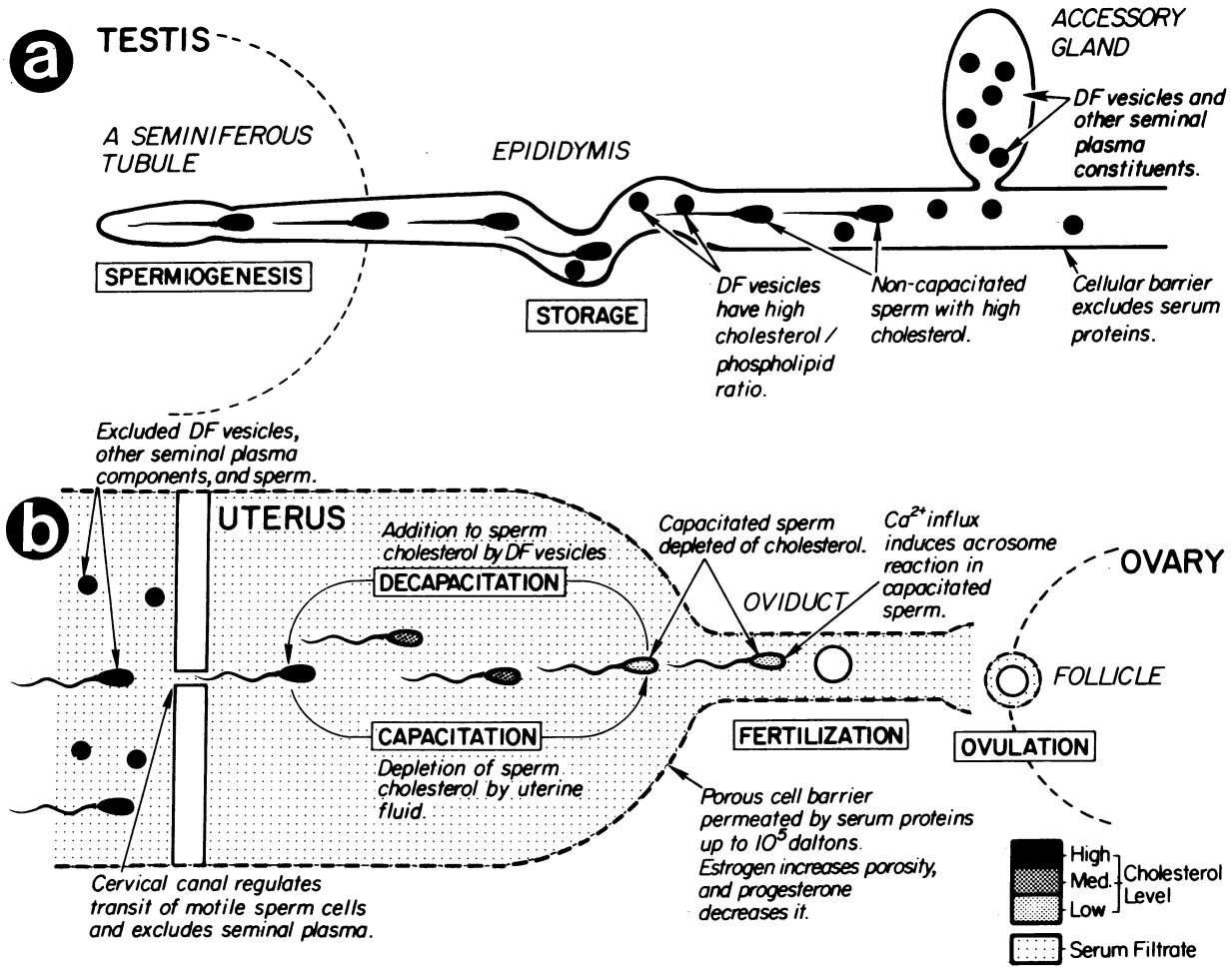

FIG. 2. Diagram showing proposed changes in sperm Chol level with spermiogenesis, sperm retention, capacitation, and fertilization in the male (*a*) and female (*b*) generative tracts of a representative mammal.

Elevations in sperm respiration accompany both capacitation and decapacitation (45) and *in utero* incubation under capacitating and noncapacitating conditions (46), suggesting that the event is incidental. Capacitation is probably independent of external Ca^{2+} concentration (47). Consequently, an energy-driven inward Ca^{2+} "pump" (48), Ca^{2+}-dependent acrosin autoactivation, and a Ca^{2+} (calmodulin complexed?) activation of adenylate cyclase (sperm phosphodiesterase seems Ca^{2+} independent) with ensuing elevations in cAMP (49) are unlikely to be directly responsible for capacitation. Mechanisms involving enzymatic hydrolysis of membrane components, such as PL, are difficult to reconcile with the reversible nature of this transformation.

Although Chol is antifusigenic under various experimental conditions, its physiological function in membranes has remained an enigma. The acrosome reaction could be the first Ca^{2+}-induced membrane fusion found to be modulated through bilayer Chol. Precisely how Chol exerts this effect is not established. However, by intercalating between PL molecules, it can act as a space-filling condensing agent. A reversible expansion of bilayer PL follows Chol removal, at temperatures above the gel → liquid-crystalline transition temperature. An asymmetric Ca^{2+}-induced condensation of this liquid-crystalline PL bilayer presumably forms unstable, transient gaps in one leaflet. The destabilized membrane may break and reanneal (50) or fuse with a neighboring membrane (51). Initiation of the acrosome reaction at regions of high charge density (52) is suggestive evidence linking Ca^{2+}-anionic PL interactions with fusion between plasma membrane and outer acrosomal membrane.

Under *in vitro* conditions, serum albumin specifically facilitates capacitation, especially after delipidation. An optimal protein/sperm Chol mole ratio of 50 was found for rat spermatozoa (53). This exceeds by 30-fold the Chol-binding capacity of albumin at physiological pH and temperature (54). There is recent evidence that PL and Chol exchange with albumin lowers the sperm Chol/PL ratio *in vitro* (17, 55), and the magnitude of this decrease is consistent with present expectations. Albumin is not essential for capacitation *in vivo*, however, as analbuminemic rats show normal fertility (56). Here, an explanation is suggested by their elevated serum α- and β-globulins, which seem to bind sperm Chol *in utero*. Recently, uterine porcine sperm were reported to show a small increase in PL (57). The estimated decrease (0.05) in Chol/PL ratio in fact agrees with that deduced (0.07) from the proposed model.

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1. Austin, C. R. (1974) *Proc. R. Soc. Med.* **67**, 925–927.
2. Chang, M. C. (1957) *Nature (London)* **179**, 258–259.
3. Davis, B. K. (1974) *J. Reprod. Fertil.* **41**, 241–244.
4. Davis, B. K. (1978) *Symp. Pharmacological Effects of Lipids*, Am. Oil Chem. Soc. Monograph No. 5 (Champaign, IL), pp. 145–157.
5. Davis, B. K. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 1867.
6. Hunter, R. H. F. & Hall, J. P. (1974) *Anat. Rec.* **180**, 594–604.
7. Austin, C. R. & Braden, A. W. H. (1954) *Aust. J. Biol. Sci.* **7**, 179–194.
8. Adams, C. E. & Chang, M. C. (1962) *J. Exp. Zool.* **151**, 159–166.
9. Mattner, P. E. (1963) *Nature (London)* **199**, 772–773.
10. Iritani, A. & Niwa, K. (1977) *J. Reprod. Fertil.* **50**, 119–121.
11. Bavister, B. D., Edwards, R. G. & Steptoe, P. C. (1969) *J. Reprod. Fertil.* **20**, 159–160.
12. Darin-Bennett, A. & White, I. G. (1977) *Cryobiology* **14**, 466–470.
13. Komarek, R. J., Pickett, B. W., Gibson, E. W. & Jensen, R. G. (1965) *J. Reprod. Fertil.* **9**, 131–136.
14. Johnson, L. A., Pursel, V. G. & Gerrits, R. J. (1972) *J. Anim. Sci.* **35**, 398–403.
15. Scott, T. W. (1973) *J. Reprod. Fertil. Suppl.* **18**, 65–76.
16. Poulos, A., Darin-Bennett, A. & White, I. G. (1973) *Comp. Biochem. Physiol. B* **46**, 541–549.
17. Davis, B. K., Byrne, R. & Bedigian, K. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1546–1556.
18. Friend, D. S. (1980) in *Membrane-Membrane Interactions*, ed. Gilula, N. B. (Raven, New York), pp. 153–165.
19. Goswami, A., Kar, A. B. & Chowdbury, S. R. (1963) *J. Reprod. Fertil.* **6**, 287–295.
20. Davis, J. S. & Alden, R. H. (1963) *Anat. Rec.* **134**, 725–738.
21. Okey, R., Bloor, W. R. & Corner, G. W. (1930) *J. Biol. Chem.* **86**, 307–314.
22. Grogan, D. E., Mayer, D. T. & Sikes, J. D. (1966) *J. Reprod. Fertil.* **12**, 431–436.
23. Misiewicz, L. (1968) *Ginekol. Pol.* **39**, 859–862.
24. Chang, M. C. (1958) *Endocrinology* **63**, 619–628.
25. Jain, Y. C. & Anand, S. R. (1976) *J. Reprod. Fertil.* **47**, 255–260.
26. Komarek, R. J., Pickett, B. W., Gibson, R. N. & Lanz, J. (1965) *J. Reprod. Fertil.* **10**, 337–342.
27. Clegg, E. D. & Foote, R. H. (1973) *J. Reprod. Fertil.* **34**, 379–383.
28. Poulos, A. & White, I. G. (1973) *J. Reprod. Fertil.* **35**, 265–272.
29. Quinn, P. J. & White, I. G. (1967) *Aust. J. Biol. Sci.* **20**, 1205–1215.
30. Fogelman, A. M., Seager, J., Edwards, P. A. & Popjak, G. (1977) *J. Biol. Chem.* **252**, 644–651.
31. Bavister, B. D. & Morton, D. B. (1974) *J. Reprod. Fertil.* **40**, 495–498.
32. Kulangara, A. C. (1976) *J. Reprod. Fertil.* **46**, 189–194.
33. Bedford, J. M. (1967) *J. Reprod. Fertil. Suppl.* **2**, 35–48.
34. Davis, B. K. (1976) *Proc. Soc. Exp. Biol. Med.* **152**, 257–261
35. Bedford, J. M. & Chang, M. C. (1962) *Am. J. Physiol.* **202**, 179–181.
36. Davis, B. K. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 1546.
37. Chernoff, H. N. & Dukelow, W. R. (1969) *J. Reprod. Fertil.* **18**, 141–144.
38. Pinsker, M. C. & Robertson, R. T. (1969) *J. Reprod. Fertil.* **18**, 175.
39. Bedford, J. M. (1972) in *Reproductive Biology*, eds. Balin, H. & Glaser, S. (Excerpta Med., Amsterdam), pp. 338–392.
40. Szumowski, P. (1961) *Fourth International Congress on Animal Reproduction and Artificial Insemination* (The Hague, The Netherlands), Vol. 3, 696–700.
41. Oliver, M. F. & Boyd, G. S. (1953) *Clin. Sci.* **12**, 217–222.
42. Singh, E. J. (1975) *Am. J. Obstet. Gynecol.* **123**, 128–132.
43. Davis, B. K. (1978) *Proc. Soc. Exp. Biol. Med.* **157**, 54–56.
44. Katz, D. F., Yanagimachi, R. & Dresdner, R. D. (1978) *J. Reprod. Fertil.* **52**, 167–172.
45. White, I. G., Rodger, J. C., Murdoch, R. N., Williams, W. L. & Abney, T. O. (1975) *Experientia* **31**, 80–81.
46. Murdoch, R. N. & White, I. G. (1967) *J. Reprod. Fertil.* **14**, 213–223.
47. Yanagimachi, R. & Usui, N. (1974) *Exp. Cell Res.* **89**, 161–174.
48. Gordon, M. (1973) *J. Exp. Zool.* **185**, 111–120.
49. Garbers, D. C. & Kopf, G. S. (1980) *Adv. Cyclic Nucleotide Res.* **13**, 251–306.
50. Gingell, D. & Ginsberg, L. (1978) in *Membrane Fusion*, eds. Poste, G. & Nicholson, G. L. (Elsevier, New York), pp. 791–833.
51. Papahadjopoulos, D., Vail, W. J., Newton, C., Nir, S., Jacobson, K., Poste, G. & Lazo, R. (1977) *Biochim. Biophys. Acta* **465**, 579–598.
52. Friend, D. S., Orci, L., Perrelet, A. & Yanagimachi, R. (1977) *J. Cell Biol.* **74**, 561–577.
53. Niwa, K. & Chang, M. C. (1974) *J. Exp. Zool.* **189**, 353–356.
54. Westphal, U. (1971) *Steroid Protein Interactions* (Springer, New York).
55. Davis, B. K., Byrne, R. & Hungund, B. (1979) *Biochim. Biophys. Acta* **558**, 257–266.
56. Nagase, S., Shimamune, K. & Shumiya, S. (1979) *Science* **205**, 590–591.
57. Evans, R. W., Weaver, D. E. & Clegg, E. D. (1980) *J. Lipid Res.* **21**, 223–228.