Stud male-originating chemosignals: A luteotrophic agent*

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The chemosignals from mating male are found to be responsible for protecting his coital partner against pregnancy failure induced by strange male or food-deprivation. The stud male pheromone not only provides luteotrophic support in female of vulnerable condition but also exerts luteotrophic effect in pregnancy-blocked females by inducing pseudopregnancy. The luteotrophic stimulus rendered by stud male to prevent pregnancy failure is mediated through the main olfactory system, and not through the accessory olfactory system, since the accessory olfactory system is primarily involved in perceiving the luteolytic stimulus produced from strange male for causing pregnancy failure. It has been shown that pericopulatory period seems to be crucial in females in the formation of stud male chemosignals, and the olfactory luteotrophic memory of stud male is further proved to be a short-term one. The precise mechanism involved in accessing and retaining the stud male chemical cues is unclear. In this brief review an attempt has been made to bring out the luteotrophic process of stud male chemosignals, the olfactory pathway and critical period to access the signals. The possible neural mechanism and neural chemistry underlying the formation and recognition of mating male chemical cues are also highlighted.

Keywords: Chemosignals, Olfactory luteotrophic memory, Pheromone, Stud male

Introduction

Exteroceptive factors such as light, temperature, food and social stimuli greatly influence mammalian reproduction. Among the social stimuli, the species-specific chemical signals, i.e., pheromones, play a significant role in reproductive physiology and behavioural modulation in several mammalian species including rodents\textsuperscript{1,5}, ungulates\textsuperscript{6,7}, proboscidae\textsuperscript{8} and primates\textsuperscript{9,10}. The stimulatory effects of male on females, such as advancing sexual maturity, induction of estrus, reduction of postpartum anoestrus and identification of estrus, are some of the important biological features of mammalian pheromones. Not only the reproductive behaviour is modulated, but the pheromonal signals interfere with other behaviours such as scent marking\textsuperscript{11,12}, individual recognition\textsuperscript{13,14}, aggression\textsuperscript{15}, mother-young interaction\textsuperscript{16}, and so on.

A number of sources for both primer and releaser pheromones viz., urine, feces, sweat, saliva, vaginal fluid, specialized scent glands, etc., have been reported.

The primer pheromonal effects, such as acceleration of puberty, mutual suppression of estrous cycle, induction of estrus in group-housed females and blockage of pregnancy in newly inseminated females\textsuperscript{17-19}, have been reported in mice but the compounds which are necessary to activate the primer pheromonal effects are only partially identified\textsuperscript{20}. Among the primer pheromonal effects, the strange male-induced pregnancy failure (the Bruce effect) appears to be a classical one but the process is found to be complicated since it involves several mechanisms which include endocrine, neurochemical and memory. An important investigation pertaining to the Bruce effect is that the strange or unfamiliar male will become ineffective in inducing pregnancy failure when the newly-mated female mouse is housed with the stud male\textsuperscript{21,22}. This shows that olfactory luteotrophic memory system plays a significant role in the female to respond to the other male. Therefore, it is a challenge to identify the neural mechanism, the process of olfactory memory formation in females during the period of mating, and retention of memory signals by females.

Though several studies pertaining to endocrine and neural mechanisms involved in the novel male blocks pregnancy have been carried out, investigations regarding the protective role of stud male on his coital partner are few. The olfactory cue produced from unfamiliar male in inducing pregnancy block causes luteolytic effect, whereas the olfactory cue released from her coital partner counteracts the pregnancy failure by stimulating the luteotrophic effect.

* Dedicated to my mentor, Prof C J Dominic, who introduced me into this exciting field of pheromone research

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In addition, the role of stud male in providing luteotrophic support at critical period against fasting-induced implantation failure and pregnancy-blocked females by induction of pseudopregnancy is noteworthy. In the present review, the protective role of stud male-originating olfactory cues on early implantation failure in mice, and the chemosensory pathway, the critical period and the possible neural mechanism in this protective role are discussed.

**Stud male protection against strange male-induced pregnancy failure**

When a newly-mated mouse is removed from the stud male and exposed to another adult conspecific male, pregnancy fails and the female returns to estrus as if mating had not taken place. It is well known that the urinary pheromones from strange males cause the suppression of hypophysial prolactin release in newly-inseminated mice. The decrease of prolactin leads to regression of corpora lutea which results in decreased progesterone level and so, the blastocyst does not implant. Investigators have used different terms like strange, alien, unfamiliar and novel males for the male used to induce the pregnancy failure in newly inseminated mouse. Whatever the case related to their experimental set up, they all differ from stud male. It is important to note that the absence of the stud male alleviates the pregnancy failure in females where the presence of stud male during the exposure of the alien male significantly reduced the implantation failure in newly-mated mouse. This clearly shows the protective role of stud male in respect to her coital partner by stimulating the luteotrophic mechanism and, thus, preventing pregnancy failure. It has been further found that contact of stud male odour is necessary for the female to exhibit the luteotrophic activity. In rodents, there is no functional corpus luteum, which is initiated for its activation following mating or vaginal stimulation. This implies that once the stud male odour initiates the corpus luteum to be functional in her coital partner through vaginal stimulation during mating, the neural mechanism is retained by olfaction and reactivated when situation warrants. However, in case of mice, the counteractive role of stud male, whether the stud male plays the same protective role as in other rodents where the corpus luteum is made functional by mating, is not known.

In contrast to the significant reduction in implantation failure in newly mated females exposed to alien males when housed with stud males, the newly inseminated females housed with a familiar male during exposure to alien male showed high rate of pregnancy failure. The familiar male was exposed to the female before mating i.e., precopulatory period. The results suggest that the protective effect of stud male on implantation is not because of the familiarity of the female with his odour cues but the female mouse identifies her coital partner through the olfactory cue imprinted during the time of mating.

A study by Milligan et al. in another rodent, vole (Microtus agrestis), provides supportive evidence that the luteotrophic stimulus of mating is retained and appears to be remembered. These authors found the corpus luteum induced by mating and destroyed by bromocriptine was activated by hormone treatment and suggest that the newly initiated corpus luteum was due to the luteotropic effect of mating for a few days. It is a general opinion that the strange male or novel male is able to initiate the pregnancy failure by suppressing the luteotropic ‘mnemonic’ activated by the stud male. Re-exposure of female to the stud male during exposure to strange male appears to protect the luteotrophic mechanism originally initiated by him.

The Bruce effect does not occur in rats. However, newly inseminated mice exposed to male rat-soiled bedding exhibited a high rate of pregnancy failure; but inseminated mice housed with female rats or their soiled bedding showed lower rate of pregnancy failure. It is interesting to note that exposure to the stud male prevented the pregnancy failure in mice induced by male rats or male rat-soiled bedding. This finding is analogous to the protective effect of the stud male on pregnancy in female mice exposed to a strange male. Therefore, it raises the possibility that the protective role of stud male against male rat-induced pregnancy failure is due to the stimulation of luteotropic activity of stud male-originating olfactory cue. It further indicates that stud male odour is capable of activating the luteotropic memory by protecting her partner irrespective of intra-specific and inter-specific social stimuli.

**Stud male protection against nutritional stress-induced implantation failure**

Reproductive processes like gestation and lactation are energetically costly for female mammals. The deleterious effects of food deprivation on reproductive functions are well documented in human and animals. The food restriction delays puberty, blocks ovulation and interrupts pregnancy and sexual behaviours in mice, rats, hamsters, guinea pigs, and other rodents.
musk shrews and cattle. Food-deprivation for 48 h during the pre-implantation period is reported to induce implantation failure in laboratory mice. However, the short-term food deprivation during post implantation period, i.e., day 7 or 8 post-coitum, does not induce pregnancy failure in mice, suggesting that the 48 h inanition after the formation of implantation is ineffective to cause luteal failure once placenta takes over the charge of gestational activity. This nutritional-stress induced implantation failure is prevented by administration of exogenous prolactin, presence of ectopic pituitary graft or drugs, such as methyl dopa, reserpine and chlorpromazine which stimulate the release of hypophysial prolactin. Since these treatments help to maintain functional corpora lutea, it is evident that failure of hypophysial prolactin release is the primary endocrine cause leading to implantation failure in food-deprived females.

The fasting-induced implantation failure is prevented by the presence of the stud male, resulting in the saving of early blastocysts during the critical period. It was further observed that the length of gestation and the litter size in the food-deprived females housed with stud males were comparable to that seen in undisturbed females. Since impairment of prolactin release appears to be the primary endocrine factor involved in the failure of implantation in food-deprived females, it is obvious that the stud male-originating chemosignals provide luteal support in stimulating the hypophysial prolactin in the female during fasting.

In contrast to the presence of the stud male, the presence of any other male does not protect the pregnancy in food-deprived females. It indicates that the newly mated female is able to remember and identify her partner. Further, it should be remembered that in contrary to the maintenance of estrous cycle in underfed female rats and mice by the exposure of any conspecific male, pregnancy will not be maintained in nutritionally-stressed mice by the presence of any male other than the stud male. This is consistent with the view that newly inseminated female is able to identify the stud male which mated with her. Larsen and Grattan have...

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**Role of stud male on induction of pseudopregnancy in pregnancy-blocked females**

Experiments were carried by Thomas and Dominic to confirm the effective role of stud male in luteotrophic activity of the female and provided unequivocal evidence in this context. Newly mated female mice either exposed to an alien male or administered with bromocriptine during the pre-implantation period exhibited implantation failure or returned to estrus on day 3 or 4 post-coitum. Re-exposure of such female mice to the stud male induced pseudopregnancy in the female mice whose pregnancies were blocked either by alien male exposure or treatment with bromocriptine. The occurrence of pseudopregnancy was confirmed by decidual cell response in both the cases. These reports indicate that the newly inseminated female mouse is able to retain the olfactory luteotrophic memory of the stud male for a certain period after mating, and reactivation of this memory by re-exposure to the stud male induces a luteotrophic effect leading to pseudopregnancy in pregnancy-blocked female (Fig. 1). This luteotrophic effect has been reported to occur in pregnancy-blocked female by re-exposure to the stud male only, not by any other male. This is consistent with the view that newly inseminated female is able to identify the stud male which mated with her. Larsen and Grattan have...

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**Fig.1—Schematic representation showing luteotrophic effect of stud male in newly inseminated female.** Newly inseminated females (NIF’s) when exposed to an alien male (A), food deprivation (F) or bromocriptine (B), pregnancy failure (PF) occurs. These females (NIF) when exposed to A and F housed with stud male (SM), there is no pregnancy failure (NPF). When NIF’s treated with bromocriptine (B) are housed with stud male (SM), pregnancy failure (PF) occurs. Those females (NIF) return to estrous cycle following exposure to A and B and housed with stud male (SM) exhibit pseudopregnancy (PP).
recently reported that mating induces increased secretion of prolactin, which is essential for maintenance of pregnancy and for adaptive changes in maternal brain in the rodent. It is thus possible to conclude that the mating induced luteotrophic ‘mnemonic’ in female is being activated by the stud male even after the termination of pregnancy.

**Failure of stud male to protect implantation failure caused by bromocriptine**

Experiments have proved beyond doubt the protective effect of stud male on pregnancy in alien male-exposed as well as in food-deprived females. However, the stud male failed to prevent the implantation failure caused by treatment with bromocriptine. A single injection of bromocriptine (0.5 mg) on day 1 post-coitum induced high rate of implantation failure in newly mated females. Since bromocriptine is a potent inhibitor of hypophysial prolactin release, by virtue of its stimulatory effect on hypothalamic dopaminergic activity, the results suggest that the stud male-originating luteotrophic stimulus is incapable of overriding the dopaminergic activity in bromocriptine-treated females. This is in contrast with the ability of the stud male to prevent implantation failure in females induced by alien male exposure or nutritional stress. In addition, stud male induced pseudopregnancy in a large proportion of female mice whose pregnancies were terminated by a single injection of bromocriptine (0.5 mg) on day 1 post-coitum. It shows that stud male odour is capable of bringing about the luteotrophic effect in female even after the termination of pregnancy caused by treatment with bromocriptine but is incapable of providing luteal support in her coital partner which received bromocriptine to block the pregnancy. It should be remembered that continuous exposure for at least 48 and 72 h is necessary for nutritionally stressed and alien male exposed female, respectively, for manifesting the implantation failure, indicating that the process is moderate and slow. Since bromocriptine is administered as a single injection, the initiation process may be strong and fast. The actual neural mechanism involved in the inability of stud male to restore bromocriptine induced pregnancy failure in mouse is not yet understood.

**Chemosensory pathways in perception of stud male pheromone**

It is firmly established that mammals have a dual olfactory system, i.e., Main Olfactory System (MOS) and Accessory (vomeronasal) Olfactory System (AOS), and both are reported to be involved in the perception of pheromones. Large body of evidence indicates that the AOS is involved in the perception of several pheromones, for instance, the pregnancy block (Bruce effect) and the acceleration of puberty (Vandenbergh effect) are caused by male pheromones, mediated through vomeronasal system.

The chemosensory pathway involved in influencing the luteotrophic effect of stud male in females is amazing. Vomeronasal organ (VNO)-ablated females housed with stud males during fasting exhibited a significant decrease in implantation failure comparable to that seen in sham-operated and intact females housed with stud males during food deprivation. This finding clearly indicates that the VNO is not involved in the perception of the stud male-originating olfactory cue that protects pregnancy in food-deprived females. On the other hand, Zinc Sulphate (ZnSO₄)-irrigated females housed with stud males during fasting showed a high rate of implantation failure. The results provide strong circumstantial evidence that the main olfactory system is a necessary pathway in the perception of the stud male-originating olfactory cue, which induces luteotrophic effect and subsequently protects the implantation in food-deprived females. Since it is generally believed that MOS is involved in the perception of volatiles, i.e., air-borne substances, the inability of stud males to protect implantation in ZnSO₄-irrigated food-deprived females implies that the olfactory cue of stud male that causes luteotrophic effect in females is likely to be air-borne. It is further demonstrated that stud males anointed with artificial scents failed to protect implantation in underfed females, suggesting that the stud male olfactory cue concerned with the luteotropic role is volatile in nature. The ability of VNO-ablated females to detect the olfactory cue emanating from stud males contrasts their inability to perceive the alien male-originating pheromone involved in implantation failure. It shows the chemosensory pathway involved in perception of olfactory cues in females for luteolytic effect from unfamiliar male and for luteotropic effect from stud male is different. It is of interest to note that the strange or unfamiliar male odour is incapable of inducing luteolytic effect in newly inseminated females when stud male is co-habitated. It is to be noted that the olfactory pathway involved in the perception of strange/alien male and stud male is entirely different; the former odour influences its
‘luteolytic effect’ through AOS whereas the latter odour influences ‘luteotrophic effect’ on his coital partner through MOS. It is obvious that the MOS is dominant over the AOS in the newly mated female as far as the luteotrophic effect/memory is concerned. It is important to note that the female is able to discriminate the chemosignals of the male which mated her and those other males, and access the luteotrophic odorants for saving their offspring. Thomas and Dominic have also found the ability of the stud males to induce pseudopregnancy in VNO-ablated coital partners whose pregnancies were terminated by treatment with bromocriptine, where as stud males are ineffective in inducing pseudopregnancy in pregnancy blocked-females made anosmic by intranasal irrigation with ZnSO$_4$ solution. The finding is strongly supported by the report that odours pertaining to mate recognition in mice are mediated through MOS and not by the AOS. Altogether, it is pertinent to conclude that the formation of olfactory luteotrophic memory of stud male in females during mating, and retention and recognition of the coital partner’s chemosignals in order to manifest luteotrophic effect in females is mediated through MOS.

In a recent report, it has been shown that pheromones from familiar and unfamiliar males have different effects on the newborn’s Accessory Olfactory Bulb (AOB) cell survival. While male pheromones increase cell survival in the female AOB, exposure to unfamiliar ones blocks the survival effect promoted by the first male pheromone exposure. The pheromones from familiar or unfamiliar males might activate discrete olfactory maps or induce distinct signal transduction cascades, resulting in different outcomes in AOB neurogenesis. This study strongly supports previous reports that unfamiliar male/strange male pheromones cause the pregnancy failure through AOS. Though the study brings out new information on cellular changes in AOS, the MOS has not been investigated in regard to exposure of either familiar or unfamiliar males. Therefore, experimental study is required in regard to both AOS and MOS simultaneously, from which only it can be inferred which of the olfactory systems mediates the perception of familiar or unfamiliar male pheromone odour. A number of pheromonal effects are mediated through MOS and not by VNO in several mammalian species. For instance, the suckling behaviour exhibited by rabbit pups in getting the milk from mother is mediated by MOS. Female golden hamster without the vomeronasal system is able to discriminate between individuals on the basis of the odours produced from male flank glands or vaginal secretion. Eisthen et al. reported that the VNO has no role in male guinea pig in responding to the chemical signals.

In the pig, the salivary pheromone facilitates the mating stance in females, which is important for coitus. Blocking of vomeronasal pathway here did not suppress the pheromonal effect in female pigs. In addition, estrus ewes stimulate luteinizing hormone (LH) secretion in the male, which is not affected by removal of VNO. In sheep, olfactory cues from the neonate are important for the establishment of maternal behaviour and selective bonding. Vomeronasal nerve transection did not disturb both the maternal behaviours and selectivity at suckling whereas anosmia induced by zinc sulphate irrigation severely affected these behaviours. These studies provide evidence for indication that some of the pheromonal communications are perceived by the MOS. It is true that MOS and AOS act independently in the perception of particular pheromonal effects. However, investigations provide circumstantial evidence that both MOS and AOS of male act together in the perception of a few pheromone responses; particularly the chemosignals released by the female during estrus are mediated through MOS as well as AOS in the mouse and the rat. The investigation showed that in male mouse (i) MOS detects estrus through volatiles released by female even from a distance, and (ii) VNO confirms the estrus by follow-up pre-copulatory behaviours like sniffing, licking and mounting indicating that accessory olfactory vomeronasal system has a synergistic role in detecting estrus and the mating.

**Critical period and memory formation**

The occurrence of critical period for the formation of memory of stud male in newly inseminated female is quite interesting. Formation and retention of the memory appear to be very important for luteotrophic effect. Failure to form this memory makes dramatic change in the stud male being treated as strange male and, hence, his olfactory cues terminate pregnancy. It was generally thought that a male other than stud male could provide such protective effect on pregnancy in alien male-exposed and food-deprived females.

A series of experiments was carried out on this aspect. A male to whom the female was exposed for maximum of 7 days before mating and for one day (i.e., 24 h) during the day of mating is considered ‘familiar’ male was used in the experiment. A familiar
male with whom the female was exposed for a prolonged period before mating was found to be ineffective in providing protection against strange male-induced pregnancy failure. However, Kumar and Dominic have reported that ‘familiar’ male with whom the female was exposed for a short period, i.e., 24 h during the time of mating was able to prevent the pregnancy failure induced in females by strange males. This indicates that the familiar male is capable of providing luteotrophic effect provided the female was cohabitated with this male during the time of mating.

Further, ‘familiar’ males, which had prolonged precopulatory stay, i.e., more than 7 days, with females, were ineffective in protecting pregnancy in food-deprived females. By contrast, ‘familiar’ males, which had a brief stay with females for only 24 h during the pericopulatory period, were as effective as the stud males in protecting pregnancy in food-deprived females. It is to be remembered that if the female is exposed to a male (stud or ‘familiar’) during this critical period she retains the memory of his odour, which enables her to recognize and respond to the odour later after mating resulting in the stimulation of luteotrophic effect. The results further underscore the fact that it is the time of cohabitation with the male, and not the duration, that is critical in the formation of the luteotrophic memory in the female. These findings strongly support the view that the pericopulatory period is critical in the formation of the olfactory luteotrophic memory in the female.

If female voles were allowed contact with stud male for only 1 h at the time of mating, there was a reduction in pregnancy failure (55%) when exposed to strange male. By contrast females which were made pseudopregnant by hormone treatment or vaginal stimulation, without stud male involvement, had luteal failure at a rate relatively high (87%) when exposed to strange male. It shows the duration of cohabitation of stud male with the female during the time of mating is important for imprinting the olfactory luteotrophic memory in female. The finding of Keverne and de la Riva showed that the female retains the memory of her coital partner provided the female is allowed to cohabit for at least 4 h immediately after mating. It suggests that a short period of exposure, particularly immediately after the time of mating, appears to be highly sensitive and critical for the female to learn about and access the luteotrophic odour.

### Duration of olfactory luteotrophic memory

The opinion concerning the duration of olfactory luteotrophic memory of the stud male by the female differs. Thomas and Dominic reported the ability of stud male to induce luteotrophic effect in pregnancy-blocked females is a maximum of 5 days post-coitum. However, other studies indicate that females retain the olfactory memory of the stud male for 30-50 days after mating. Acharya and Dominic provided convincing evidence that the mating recognition of the stud male for stimulating the luteotrophic support in female remains up to 7 days after mating. They further investigated that each pregnancy-blocked female was mated with the (alien) male that induced failure of the first pregnancy and then housed with simultaneous access to the excreta of another alien male and that of the first stud male and proved that excreta of the first stud male did not protect the failure of the second pregnancy. It indicates that female mice do not show luteotrophic response when exposed to the odour of coital partner after 7 days following mating. It is also obvious from the above study that the male who inseminated her will be able to protect her pregnancy through odour for a maximum period of seven days after coitus.

Brennan has reported that the formation of stud male memory primarily involves the ineffectiveness in blocking the pregnancy by urinary signals from that mated male rather than its effective participation in preventing the pregnancy failure. Since in both the circumstances, whether the ineffectiveness to block pregnancy or protect the pregnancy failure by chemosignals of stud male, the formation of stud male memory is related to luteal function, the physiology involved in the recognition of mating does not make much difference. It is to be remembered that nature would not allow the male to cause block of pregnancy caused by him. From the ecological point of view, the pregnancy is protected by the coital partner if he is present in the vicinity of the female. Moreover, the protection rendered by the stud male chemosignals against pregnancy failure caused by environmental cues such as social odours and nutritional stress is really significant and value-based. The effective role of stud male chemosignals in the aspects of pregnancy protection in mice against other environmental cues remains to be addressed.

It may be asked why the female does not show luteotrophic response following exposure or re-exposure after day 8 post-coitum, to the stud male odours in
spite of the stud male odour having been retained by the female. The answer is to be sought in the fact that there are two types of memories which are actually formed in respect of mating recognition. The simple mating recognition is one in which memory of the stud male may persist for about 30 days or even more after mating \(^{84,86}\). This memory probably serves in discriminating the newer male from the previous partner for selection of mating. It may be called ‘long term memory’ or ‘simple memory’ of mating recognition. The second memory is concurrently formed in females during mating in which the newly mated female exhibits luteotrophic response only when exposed to her coital partner. This ‘olfactory luteotrophic memory’ lasts only about 7 days or less. In this case, a particular odour from the stud/mating male is genuinely involved in the specific/critical period. The characteristics of this memory are formed by the coital partner, and might fade following a subsequent mating. Therefore, this olfactory memory may be described as ‘short term memory’, or which may also be called ‘olfactory luteotrophic memory’ in the context of mating recognition.

**Neural mechanisms and neural chemistry in memory formation**

Despite several studies pertaining to the formation of olfactory luteotrophic memory of mating male’s chemosignals in female mouse, the precise role of neural mechanism and neural chemistry on the perception and reactivation of study male luteotrophic memory is not yet clearly understood. Detailed investigations about AOS and its neural connections in the olfactory recognition of study male signals have been carried out \(^{87-93}\). On the other hand, investigations relating to the main olfactory pathway and its neural connections for the access and recognition of study male signals are very limited \(^{62,64}\). The pregnancy-blocking effect of chemosignals of strange/alien male are mediated through the AOS. Therefore, several studies have been directed towards AOS. Though these studies provided considerable evidence in respect of neuro-chemicals involved in the pregnancy blocking effects, the exact role of neural mechanism and neuro-chemical changes that occur during the process of learning and recognizing the coital partner’s signals are not clearly defined.

Reports indicate that medial amygdala is one of the principal brain regions for the activation of the male odours through neuroendocrine mechanism \(^{81}\). Even though both the main olfactory bulb (MOB) and accessory olfactory bulb (AOB) project in to the amygdala, they terminate in adjacent non-overlapping areas \(^{94,95}\). Therefore, it is obvious that the two olfactory pathways do not have even indirect connection for olfactory processing. It is found that hippocampal region is involved in olfactory learning and recognition in mice \(^{86}\). However, the role of hippocampal region in the formation and reactivation of stud male memory in mouse is yet to be identified. Nevertheless, study conducted by Demas et al. \(^{97}\) in prairie vole showed that a greater proportion of amygdala-lesioned females displayed pregnancy failure following re-exposure to coital partner as compared to hippocampus-lesioned voles. The amygdala appears to be an important region for the formation of olfactory memory about the coital partner. The reports concerning to neuroanatomy for olfactory learning and recognition are ambiguous. Further studies adopting appropriate techniques may clarify this issue.

The effect of neurochemicals on the formation of memory about stud male chemosignals in female is inconsistent. The formation of stud male’s luteotrophic memory is based on the high level of noradrenaline (NA) in female AOB immediate after mating \(^{81,98}\). Inhibition of NA in the AOB either before mating or following local infusion of an alpha noradrenergic antagonist during the critical period after mating prevents the formation memory about the coital partner \(^{99,100}\). The increase of NA seems to be important for the formation memory about the coital partner. The other neurochemicals such as glutamate, aspartate and gamma-aminobutyric acid (GABA) are thought to be the ones mainly involved in the activation of AOB during luteotrophic memory formation. Surprisingly, the results obtained by Brennan et al. \(^{90}\) are contradictory in the above findings. These authors found no significant changes in glutamate, aspartate or GABA during the critical period after mating.

Another neurochemical that may be considered as a key candidate in the context of olfactory learning and memory is nitric oxide (NO). The modulatory role of NO in the release of glutamate, noradrenaline, GABA and glycine is evident \(^{101-103}\). Local infusions of the nitric oxide synthase inhibitor, L-N-nitroarginine, into the olfactory bulb during the critical period of memory access effectively suppressed the NO synthase release but did not prevent the memory
ligands are found to be effective in blocking the low molecular weight compounds having volatile overwhelming research for the last four decades. The chemosignals in this context despite an system. Hence, it appears to be a great task to identify interlinked with olfactory luteotrophic memory specific chemical cue to manifest the effect but it is yet been successful. It is not only involved in the providing protection against pregnancy failure has not causing pregnancy failure or from stud males chemosignals produced either from strange males in male mouse urine, identification of the cycle and puberty acceleration (Major urinary protein-major histocompatibility complex (MHC) interaction and individual recognition)

Nature of the stud male odour and individual recognition (Major urinary protein-major histocompatibility complex (MUP-MHC) interaction and individual recognition)

Eventhough pheromones that modulate estrous cycle and puberty acceleration have been identified in male mouse urine, identification of the chemosignals produced either from strange males causing pregnancy failure or from stud males providing protection against pregnancy failure has not yet been successful. It is not only involved in the specific chemical cue to manifest the effect but it is interlinked with olfactory luteotrophic memory system. Hence, it appears to be a great task to identify the chemosignals in this context despite an overwhelming research for the last four decades. The low molecular weight compounds having volatile ligands are found to be effective in blocking the pregnancy but the nature of the volatiles causing the luteolytic effect is not reported.

Odour produced in conspecifics varies from individual to individual. Moreover, expression of individual odour signal is presence of major urinary proteins (MUPs), which are found in very high concentrations in the urine of male mice. The MUPs are reported to be highly variable from individual to individual, and the polymorphic nature of MUP has the capacity to render individual ownership signals. This concept was further supported by Hurst et al. that MUP pattern is responsible for individual recognition in male’s scent mark and countermarking response. If such urinary proteins are involved in individual recognition, there would be a possibility that the polymorphism of urinary proteins has a role in recognition of stud male signals. However, it is doubtful whether the stud male urinary protein acts individually or binds with a specific volatile for processing the olfactory luteotrophic memory in newly mated females.

Another possibility of the individual odour types in mice is very much dependent on polymorphic genes, particularly the genes of major histocompatibility complex (MHC). It has also been found that H2 locus genes specifically influence the recognition of stud male chemosignals in the context of pregnancy block. MHC-associated odours appear to be the low molecular weight components of urine that are bound and brought to the environment by urinary proteins. However, it is unpredictable whether the urinary proteins involved in individual recognition are MUPs or fragments of MHC proteins in urine. Thus, both MUP and MHC are highly polymorphic multigene complexes that give rise to individual differences in mouse urinary chemosignals. It has been hypothesized that the two highly polymorphic multigene complexes having separate chromosomal identity may act synergistically to release the individual volatile signal in urine. Presence of any chemical messages in body secretion is a property of the particular individual and can be used for inter-individual communication. Therefore, it would be interesting to investigate the role of MHC-MUP system in the release of mating male chemosignals in the aspect of recognition of the coital partner to provide luteotrophic effect.

Conclusion

The physiological importance of mating male signals in providing protection to pregnancy failure caused by strange male or inanition, and inducing
pseudopregnancy in pregnancy-blocked females provides unequivocal evidence for a luteotropic agent. The findings show that the newly mated female mouse is able to recognize the coital partner as an individual and its olfactory luteotropic memory is short-lived. Further, the chemosignals produced from mating male in influencing the luteotropic effect are distinct from those chemosignals released from strange male causing luteolytic effect in newly mated females as far as the chemosensory pathways are concerned. Some sort of memory is formed during mating, but the exact mechanisms by which the stud male chemosignals regulate the access and reactivate the luteotropic activities are not known. Since MOS is primarily involved in the perception of mating male odour, it is necessary to pay attention to MOS to unravel the neural mechanisms involved in this context. The formation and maintenance of olfactory memory of an individual recognition appears to be crucial and complex, and remains a challenge to investigate.

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