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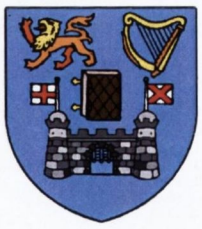
Reproductive Seasonality of the Male and Female Eurasian Badger, *Meles meles*

A dissertation submitted to the University of Dublin for the degree of Doctor of Philosophy

Lynsey Stuart

2010



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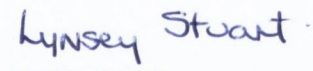

Lynsey Stuart

Date: 19th April 2010

SUMMARY

The ecology of the Eurasian Badger (*Meles meles*) is highly diverse; varying greatly between geographical regions, populations and even adjoining groups. It has been argued that mating systems result from individual strategies rather than being an evolved feature of each species. Therefore, despite close geographical proximity of populations within Europe, variations in population dynamics may greatly influence the reproductive strategy and potential of different populations. This study provided an unparalleled opportunity to combine annual morphological, histological and hormonal data from both sexes. It provided a uniquely comprehensive picture of reproductive seasonality in the badger and allowed comparisons to be made with previous studies from the south-west of England and Europe.

The nationwide control programme of reactive badger removal, adopted by the Department of Agriculture, Fisheries and Food (DAFF), provided an excellent opportunity to conduct a detailed analysis of the reproductive biology of the badger in Ireland. Badgers were obtained from a geographically wide range during all months of the year. Therefore, it was possible to describe the reproductive seasonality of the male and female badger in full, utilising a large sample size. The reproductive tracts were obtained from post mortem examinations of the badger. Both gross examination and in-depth histological examinations were carried out on all tissues; assays were conducted to determine individual levels of reproductive hormones. A number of demographic measurements were also taken to help with identifying, trends or varying population dynamics within the study population.

The badger population in Ireland may be one of low-medium density consisting of small social groups, which display high levels of inter- and intra-group tolerance. A comparison of male and female badgers suggests that each has adopted a very different breeding

strategy. The majority of males show a breeding pattern that is reminiscent of a seasonal breeder, with high fertility associated with the early oestrous cycles in the female and declining fertility for the remainder of the year. Males that remain fertile for longer periods may increase their chances of paternity during later matings. By contrast the female has adopted a strategy whereby they are lactating during a period of nutritional abundance, and cubs are also weaned during favourable conditions. This is achievable due to the long period of delay employed by this species. Badgers are polyoestrous, having continued oestrous cycles throughout the breeding year. This provides replacement or additional blastocysts, which increases the probability of successful implantation at the end of the period of diapause. Furthermore, continued oestrous cycles may increase the probability of superfeation and polyandry, leading to increased female fitness and possibly cub survival. This female strategy may also provide additional corpora lutea, which act as a source of progesterone, necessary for maintenance of the blastocyst, implantation and gestation. Failure at the fertilisation stage of the reproductive cycle was responsible for the greatest losses to reproductive potential; with approximately 40% of sexually mature females breeding successfully.

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I would like to dedicate my thesis to:

Eileen Stuart, Alfred & Antoinette Regan, Harry Stuart and Max Stuart

"What cannot be achieved in one lifetime
will happen when one lifetime is joined to another."

-Harold Kushner-

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1 INTRODUCTION

"If we knew what it was we were doing, it would not be called research, would it"

-Albert Einstein-

The following introduction is composed of two sections: social system and reproduction. The section on social system will provide a brief outline of the social organisation of the Eurasian Badger (*Meles meles*), providing a context for the development of the reproductive strategy utilised by this species. The section on reproduction will detail the events of the reproductive cycle, and the occurrence of reproductive failure and suppression within the population. Finally, the background, contribution, and objectives of this study will be summarised.

1.1 Social System

1.1.1 Group Formation

The badger exhibits a highly flexible social system. Group size ranges from pairs and small family groups in low density areas of Scotland and continental Europe (Scotland: Kruuk & Parish, 1982, Spain: Revilla & Palomares, 2002, Switzerland: Do Linh San *et al.*, 2007, Poland: Kowalczyk *et al.* 2003), where group size may be limited, to large inter-sexual groups of up to 30 individuals (Johnson *et al.*, 2000, Neal & Cheeseman, 1996, Rogers *et al.*, 1997, Stewart *et al.*, 1997a, Tuyttens *et al.*, 2000b). The Resource Dispersion Hypothesis (RDH) is often used to describe group formation in the badger

(Kruuk, 1978, MacDonald, 1983). According to the RDH, groups may form where resource availability is such that the smallest economically defendable territory for a pair of animals may also sustain additional individuals (Johnson & MacDonald, 2001, Johnson *et al.*, 2000, MacDonald, 1983). Badgers may be considered to form spatial groups rather than social groups (MacDonald, 1983). These groups may occasionally display social interactions which are behaviourally important. However, there is no compelling evidence that co-operative group living *per se* provides any direct benefit, with group members not co-operating in foraging, caring for young or defending against predators (Kruuk, 1989, Neal, 1986, Woodroffe & MacDonald, 1992, Woodroffe & MacDonald, 2000). Therefore, resource dispersion, rather than the behavioural benefits of group living may be responsible for group formation (Johnson & MacDonald, 2001, Johnson *et al.*, 2000, Johnson *et al.*, 2001, MacDonald, 1983). The RDH states that the spatial dispersal of the resources should determine territory size, with group size being determined, independently, by the abundance of the resource (Do Linh San *et al.*, 2007, Johnson *et al.*, 2000, Johnson *et al.*, 2001, Kowalczyk *et al.*, 2003, Kruuk & Parish, 1982, MacDonald, 1983).

Various resources have been suggested to be the most influential in group formation: food (da Silva *et al.*, 1993, Do Linh San *et al.*, 2007, Hofer, 1988, Kowalczyk *et al.*, 2003, Kruuk & Parish, 1982), distribution of setts or sett sites (Doncaster & Woodroffe, 1993, Roper, 1993), habitat type (da Silva *et al.*, 1993, Feore & Montgomery, 1999, Hammond *et al.*, 2001, Hofer, 1988), and/or the presence of possible mates (Johnson *et al.*, 2000, MacDonald, 1983, Tuyttens *et al.*, 2000a). The relative importance of these resources may vary temporally (Do Linh San *et al.*, 2007, Feore & Montgomery, 1999, Revilla & Palomares, 2002), between populations (da Silva *et al.*, 1993), or between the sexes (Kowalczyk *et al.*, 2003, Revilla & Palomares, 2002).

Enlarged groups, and therefore densities, such as those described in the south-west of England represent one extreme end of the range of social patterns (Cresswell *et al.*, 1992, Johnson *et al.*, 2000) and may be influenced by food abundance (Johnson *et al.*, 2000, Kowalczyk *et al.*, 2003), mean annual temperature (Kowalczyk *et al.*, 2003), sett availability (Doncaster & Woodroffe, 1993, Kowalczyk *et al.*, 2003, Roper, 1993),

climate change (MacDonald & Newman, 2002) and/or the lack of predators and human influences (Kowalczyk *et al.*, 2003). Earthworms are reported to be the favoured prey of the badger in the UK (Kruuk, 1978) and are in super-abundance in parts of the south-west of England (Johnson *et al.*, 2000). In these areas mean annual temperatures, which are thought to be becoming milder owing to climate change, lead to high food availability (Kowalczyk *et al.*, 2003, MacDonald & Newman, 2002). If sett numbers are limited and food availability is high, further increases in density may only be achieved by creating larger social groups (Kowalczyk *et al.*, 2003). It is possible that climate change may further influence densities by reducing the amount of energy required for thermoregulation, leading to increased survival and breeding rates (MacDonald & Newman, 2002)

Sociality within this species is considered to be in the relatively early stages of evolution (Kruuk, 1989, MacDonald *et al.*, 2002b). Badgers belonging to large social groups show little evidence of a stable or linear dominance hierarchy (Kruuk, 1978, MacDonald *et al.*, 2002b). From feeding studies it appeared that there was no formal organisation within the group. Dominance varied between situations based on original possession of the resource and motivation (MacDonald *et al.*, 2002b). Similar contest behaviours were observed during competition for oestrous females and during intra-group encounters (MacDonald *et al.*, 2002b).

1.1.2 Territoriality

The majority of faeces are placed at defined locations known as latrines. Latrines located near to the sett (Kruuk, 1978) and within the group territory are referred to as hinterland latrines (Roper *et al.*, 1993). Latrines known as boundary latrines are placed along territorial boarders, and vary in abundance relative to the presence of neighbouring ranges (Kruuk, 1978). Kruuk (1978) first suggested that boundary latrines may be shared and may be used to demarcate territorial boundaries by providing olfactory communication between neighbouring groups. Defecation is associated with anal gland secretions, which are group-specific and can present olfactory cues to resident and neighbouring group members (Davies *et al.*, 1988). Sniffing activity was seen during every visit to the latrine

and sometimes was the only behaviour observed, reinforcing the importance of latrines as sites of remote olfactory communication (Stewart *et al.*, 2002). In addition to providing an olfactory cue, faecal volume at shared boundary latrines may create a visual cue which also promotes range exclusion. It is considered an honest signal that relays the likelihood of encounter and/or depletion of resources within the neighbouring range. Faecal volumes were found to be matched by neighbouring groups, and higher quantities were placed at latrines closest to areas of importance to further discourage intrusion (Stewart *et al.*, 2001).

Boundary latrines, while providing a considerable group benefit, are maintained at little cost to the individual (Stewart *et al.*, 2001). Males are the main source of maintenance of boundary latrines with females predominantly using hinterland latrines (Roper *et al.*, 1993, Stewart *et al.*, 2002). This may relate to the function of the latrines, with males using boundary latrines to deter intrusion by neighbouring males, thereby guarding resident females (MacDonald *et al.*, 2004). Moreover, activity at boundary latrines tends to be greatest during the reproductive season (Revilla & Palomares, 2002, Roper *et al.*, 1993). Hinterland latrines tend to be in close proximity to the main sett and current feeding sites, two areas of important resources. Therefore, during seasons of low food abundance activity is seen to increase at hinterland latrines (Revilla & Palomares, 2002). In general, latrine activity increases in frequency relative to the current importance of the boundary or area (Stewart *et al.*, 2001).

There has been much debate about the importance of density in determining levels of territoriality within a population. It is possible that the use of border latrines and range exclusion may only occur when densities exceed a certain threshold (Do Linh San *et al.*, 2007). Low density and moderately dense populations have often been reported to exhibit low levels of territory demarcation (Do Linh San *et al.*, 2007, Kruuk & Parish, 1982). The low resource density relative to forager densities found in these populations may result in an increase in range overlap (Do Linh San *et al.*, 2007, Kruuk & Parish, 1987, Roper *et al.*, 1986), and a serious reduction in forager density could result in the breakdown of range exclusion (Stewart *et al.*, 1997a). Such increases in range overlap between neighbouring groups have been observed when group size was experimentally reduced.

However, another study which compared a number of populations found that social groups occupied discrete territories regardless of the density level of the population (Feore & Montgomery, 1999). Furthermore, at low population densities in continental Europe, badgers appeared to form stable territorial groups with the main latrines being located inside rather than along borders (Do Linh San *et al.*, 2007, Kowalczyk *et al.*, 2003, Revilla *et al.*, 2001). Therefore, it may not be the presence of territory demarcation that varies with density, but the pattern of demarcation (Do Linh San *et al.*, 2007, Hutchings *et al.*, 2002).

In high density populations faeces were predominantly left at boundary latrines by a majority of group members (Hutchings *et al.*, 2002). Conversely, hinterland latrines and temporary defecation sites spread throughout the group home range and on badger paths are of greater importance to low density populations (Do Linh San *et al.*, 2007, Hutchings *et al.*, 2001). It is this neglect of boundary latrines that has led many bait marking and latrine activity studies to conclude that low density populations are non-territorial (Revilla & Palomares, 2002). High density populations are capable of maintaining boundary latrines at the borders of neighbouring territories due to the high number of group members relative to territory size (Revilla & Palomares, 2002). Moreover, the rigorous demarcation of territory boundaries may be necessary to defend resources which are often in short supply at high densities (Feore & Montgomery, 1999, Rogers *et al.*, 2003). Low density populations may reflect unfavourable habitats with few sett sites and less reliable food resources, requiring territories to be larger. This makes it more efficient to concentrate marking behaviour to hinterland latrines located at the sett and/or within the most used areas, thereby excluding intruders from areas of most activity rather than demarking the extent of the territory (Feore & Montgomery, 1999, Revilla & Palomares, 2002, Rogers *et al.*, 2003). Therefore, although badgers were still found to be territorial in low density populations, the system of territory demarcation appears to be more flexible (Revilla & Palomares, 2002).

Kruuk (1978) described badgers as being highly territorial and reported that badgers appeared to avoid entering neighbouring ranges (Kruuk, 1978). The theory that badgers

defend an exclusive home range has been supported by the presence of latrines at territory boundaries and areas of significance (Stewart *et al.*, 2002). However, badgers from different groups have been observed meeting at boundary latrines and within neighbouring territories without evoking aggression (Stewart *et al.*, 2002). Therefore, the function of boundary latrines may not be a form of "Active Territorial Defence" (ATD), involving aggressive defence of territories from neighbouring intrusion but rather one of spatial partitioning as was proposed by the "Passive Range Exclusion" (PRE) hypothesis (Stewart *et al.*, 1997a).

PRE has been presented as an alternative or additional function of boundary latrines, which may work in conjunction with active defence strategies or act to reinforce them. According to the PRE hypothesis, boundary latrines may not indicate the limit of a group's range; it may instead serve as an information site for the two groups. The latrine size and associated olfactory cues provide a guide to resource depletion within the neighbouring range, thus permitting a mechanism for optimal foraging. From radio-tracking observations it was evident that there was a degree of overlap between neighbouring ranges at the boundary area, probably resulting from both groups utilising available resources (Stewart *et al.*, 1997a). Feeding incursions may be assessed daily from the volume of faeces deposited at boundary latrines. If resources are low, boundary latrines that are irregularly used by neighbouring groups may be disregarded; resulting in frequent incursions and eventual reconfiguration of the boundary between groups (Stewart *et al.*, 2001).

1.1.3 Aggression

It has been argued that badgers display a substantial level of territoriality and can be extremely aggressive towards individuals from neighbouring groups (Kruuk, 1989). Aggression occurs both within (intra-group) and between (inter-group) groups, as was witnessed by video footage of the Wytham Wood population (Oxfordshire, south-west England) (Kruuk, 1978, MacDonald *et al.*, 2004, Stewart *et al.*, 1997b). The majority of conflicts may occur between groups rather than within groups. Escalated fights were mainly observed, not at the sett, but at boundary areas where neighbouring ranges overlap

(Christian, 1995, MacDonald *et al.*, 2004). However, field based feeding experiments provided no evidence of intra- or inter-group hierarchies, with no one individual monopolising feeding sites or displaying dominance. Size may be important in determining dominance but it was not a reliable predictor of avoidance or victory during aggressive interactions (MacDonald *et al.*, 2002b).

The frequency and location of bite wounds were found to vary with age and sex, however the variation between sexes was found to be very small. Bite wounds were more common in sexually mature adults than in juveniles and more common in males than in females (Cresswell *et al.*, 1992, Delahay *et al.*, 2006b, MacDonald *et al.*, 2004). Males were 1.5 times more likely to have bite wounds than females (Delahay *et al.*, 2006b). Males were also more likely to have multiple wounds which were mainly concentrated on the rump, while bite wounding in females and cubs was predominantly located on the head (Delahay *et al.*, 2006b, Neal & Cheeseman, 1996). Inter- and intra-group aggression between males competing for breeding opportunities and territory may be responsible for the variation in bite wound frequency observed between the sexes (Cresswell *et al.*, 1992, Delahay *et al.*, 2006b, Neal & Cheeseman, 1996, Woodroffe *et al.*, 1993). Females may tend to avoid escalated aggression during fights, as only minor wounds were found in the majority of cases (Cresswell *et al.*, 1992, MacDonald *et al.*, 2004). For females, the higher frequency of head wounds may be received within the sett while defending their cubs from other group members, thereby reducing pre-emergence cub mortality (Cresswell *et al.*, 1992, Neal & Cheeseman, 1996). Moreover, it has been reported that breeding females were involved in the greatest levels of antagonism (Cresswell *et al.*, 1992, Kruuk, 1989).

There has been no general consensus regarding the seasonality of bite wounding; some authors have suggested that there is no seasonal pattern in wounding rates (MacDonald *et al.*, 2004), while others have observed a pattern, but have reported different timings for the seasonal peaks (Cresswell *et al.*, 1992, Delahay *et al.*, 2006b). Regardless of the timing, both studies considered breeding behaviour to be responsible for the increase in incidence of bite wounding, the timing of which can vary between years (Delahay *et al.*,

2006b). The peaks in bite wounding frequency were associated with a greater number of extraterritorial excursions by competing males (Cresswell *et al.*, 1992, Delahay *et al.*, 2006b, Woodroffe *et al.*, 1993), and the presence of newly born cubs, which may require increased defence by the females (Cresswell *et al.*, 1992, Delahay *et al.*, 2006b).

It is unclear how density affects bite wounding behaviour. One study in Woodchester Park (Gloucestershire, south-west England) (Rogers *et al.*, 2000), conducted during a period of increasing badger density, observed a significant decrease in the incidence of bite wounds. They concluded that there had been an alteration in the social structure of the population which resulted in less overlap between territories and hence less aggression. A similar study carried out in Wytham Woods reported a three-fold population increase during the observation period (MacDonald *et al.*, 2004). Unlike the response found at Woodchester Park, the social structure of the population was unable to alter. Although there was no evidence for an increase in severity in bite wounds in females, with increased group size, both frequency and severity increased for males. It appeared that it was the number of males within a group rather than the number of individuals that influenced the increase in bite wounding rate. In addition, the frequency of bite wounding among males also increased when there was an increase to the size of neighbouring groups (MacDonald *et al.*, 2004). Similarly, when bite wounding frequency was studied a number of years later in Woodchester (Delahay *et al.*, 2006b), the population was believed to be at carrying capacity. During this period, food availability was most likely reduced, resulting in greater levels of competition and aggression both between and within groups. Consequently, there was an increase in the incidence of bite wounding (Delahay *et al.*, 2006b). It is likely that a number of factors, operating at local levels, contribute to this variation in bite wounding patterns (Delahay *et al.*, 2006b).

Individuals from adjoining groups were often seen foraging close to each other at boundary areas where their ranges overlapped without evoking aggression and even during food shortages individuals were found to feed in the same patch (MacDonald *et al.*, 2002b, Stewart *et al.*, 2002). Furthermore, resident group members appeared not to co-operate in patch defence behaviour despite out-numbering intruders at artificial feeders (MacDonald *et al.*, 2002b). During peaceful encounters, individuals either retreated or

performed greeting behaviour (Kruuk, 1978, Stewart *et al.*, 2002) and aggressive or fearful postures were not regularly evoked by detection of neighbouring scent marks (Stewart *et al.*, 2002).

Rather than evading detection, there must be a degree of tolerance between some neighbouring groups, thereby allowing intrusions to occur with limited aggression (MacDonald *et al.*, 2002b, Stewart *et al.*, 1997a). Possible explanations for such behaviour may be: a high degree of relatedness between adjoining groups (da Silva *et al.*, 1994); the area of intrusion may not require defending (MacDonald *et al.*, 2002b); the individuals involved may not be antagonistic owing to sex, age, or status (Feore & Montgomery, 1999, MacDonald *et al.*, 2004); and/or territoriality levels may be lower based on season (MacDonald *et al.*, 2004).

1.1.4 Communication

Badgers use subcaudal gland secretions in the form of scent-marking at latrines and allo-marking of conspecifics to transmit information at an intra- and inter-group level (Buesching *et al.*, 2002, Buesching *et al.*, 2003, Stewart *et al.*, 2002). Subcaudal scent-marking, defecation, and digging and scuffing were observed at latrines and appear to function in the transfer of information (Stewart *et al.*, 2002). Scent-marking and defecation may be used in inter-group communication and digging and scuffing may convey reproductive condition during the breeding season (Stewart *et al.*, 2002). In addition to group-specific information, subcaudal scent-marks may provide information about the sex, age, body condition, and reproductive status of the depositing individual (Buesching *et al.*, 2002). Subcaudal secretions vary in colour and volume according to sex, season, and condition (Buesching *et al.*, 2002, Buesching *et al.*, 2003). Scent-marking activity increases in both sexes during the breeding season (Roper *et al.*, 1986). When associated with boundary latrines, the presence or absence of these subcaudal olfactory cues may be exploited by dispersers wishing to join neighbouring groups, or attempting extra-group matings (Stewart *et al.*, 2002). It is possible that extra-group mating may even be encouraged by such cues (Stewart *et al.*, 2002).

Males have higher secretion volumes leading to higher scent-marking activities (Buesching *et al.*, 2002) and are seen to visit boundary latrines more often than females (Stewart *et al.*, 2002). This would be expected if males were involved in communicating the presence of active mate guarding to non-resident males (Roper *et al.*, 1986). However, these visits may also be attempts to receive information regarding the reproductive status of neighbouring females, which may explain why females are involved in visits to boundary latrines (Stewart *et al.*, 2002).

Subcaudal allo-marking of group members can be either mutual, where two individuals press their subcaudal glands together, or sequential, where one individual marks the body of another (Buesching *et al.*, 2003, Kruuk, 1978). These two forms of marking occur at their highest rates during the breeding and cub-rearing season. As with scent-marking, males partake in more frequent allo-marking activities than females (Buesching *et al.*, 2003). Mutual allo-marking may involve the transfer of bacteria between subcaudal pouches of individual group members, resulting in a recognisable group odour which aids group cohesion (Buesching *et al.*, 2003). Sequential allo-marking, which occurs at far higher frequencies than mutual allo-marking, is thought to distribute the recognisable group odour and, as with scent-marking, may advertise individual-specific information such as sex, age, body condition and reproductive status (Buesching *et al.*, 2003).

1.1.5 Dispersal

Overall dispersal within this species may be limited (Pope *et al.*, 2006). Movements may be permanent or temporary, in the form of visits, it has been reported that individuals may return to their original group following months or even years away (Cheeseman *et al.*, 1988, MacDonald *et al.*, 2008, Rogers *et al.*, 1998, Roper *et al.*, 2003). The process of dispersal may be gradual and involve a transitory period where individuals spend time in both their natal set and the one into which they are emigrating (Roper *et al.*, 2003)

Dispersal rates appear to vary with population density. High density populations show high levels of social stability and infrequent permanent dispersals, with short term movements being of greater importance, while lower density populations show increased

rates of dispersal and movement (Cheeseman *et al.*, 1988, Kruuk, 1978, MacDonald *et al.*, 2008, Rogers *et al.*, 1998, Woodroffe *et al.*, 1993). Males and older individuals tend to move more between groups (Cheeseman *et al.*, 1988, MacDonald *et al.*, 2008, Revilla & Palomares, 2002, Rogers *et al.*, 1998, Woodroffe *et al.*, 1993). However, there may be no sex difference in permanent dispersals, although this does appear to vary among populations (MacDonald *et al.*, 2008). Individuals that disperse, either temporarily of permanently, may move alone or as single sex groups to live either, most commonly, in neighbouring groups or in distant groups (Cheeseman *et al.*, 1988, MacDonald *et al.*, 2008, Rogers *et al.*, 1998, Roper *et al.*, 2003, Woodroffe *et al.*, 1993). It has been reported that the distance travelled by dispersers does not vary with sex (MacDonald *et al.*, 2008).

Both temporary and permanent movements by males may be a mechanism for avoiding inbreeding (Woodroffe *et al.*, 1993) and/or maximising mating opportunities (Christian, 1995), with the timing of most male movements coinciding with the peak in female receptivity (Cheeseman *et al.*, 1988, MacDonald *et al.*, 2008, Revilla & Palomares, 2002). The size of the group to which males disperse permanently varies between populations, but the groups tend to have greater numbers of females (MacDonald *et al.*, 2008, Rogers *et al.*, 1998). Males that disperse from the natal group tend to be larger, have higher testosterone levels and are considered to be sexually active for greater periods (Rogers *et al.*, 1998, Woodroffe *et al.*, 1997, Woodroffe *et al.*, 1993). Although dispersal is rare in females, it could occur in response to intra-sexual competition for resources and/or breeding opportunities (Roper *et al.*, 2003, Woodroffe & MacDonald, 1995b, Woodroffe *et al.*, 1993). Females generally moved to smaller groups (Rogers *et al.*, 1998, Woodroffe *et al.*, 1993) and weak evidence suggests that they move to groups containing more males (MacDonald *et al.*, 2008). However, females may not be reproductively successful post dispersal (MacDonald *et al.*, 2008). As with males, female badgers partake in temporary visits, presumably to increase extra-group mating opportunities. The frequency of such visits was shown to be particularly high in late summer/autumn, especially in September (MacDonald *et al.*, 2008, Woodroffe *et al.*, 1993). Extraterritorial mating and reduction in inbreeding may not require permanent dispersal by individuals. In Wytham woods, both

sexes were reported to range beyond their social group boundaries, visiting different social groups; this behaviour was more common than individuals permanently dispersing (MacDonald *et al.*, 2008)

The costs associated with dispersal may vary with social stability and availability of resources within a particular population. Mate guarding behaviour has been observed in male badgers, with resident males actively defending oestrous females within their territory (Christian, 1995). However, its importance as a behaviour may vary both seasonally and between populations, related to levels of philopatry and the mating strategy adopted by the males (Woodroffe *et al.*, 1997, Woodroffe *et al.*, 1993). In some high density populations where dispersal occurs, territoriality and mate guarding may be relatively high. Evidence showed that immigrant males were victims of a higher level of scars and broken canines and were in worse condition than residents. However they may have benefited by achieving higher levels of intra-group paternity (da Silva *et al.*, 1994, Woodroffe & MacDonald, 1995a, Woodroffe *et al.*, 1993). As a result, males appeared to be more opportunistic in these populations, waiting in their natal groups until territories became available due to movement or death of the resident male (Woodroffe *et al.*, 1993).

Alternatively, aggression towards dispersers may be low. One high density population showed no aggressive responses towards the disperser from either members of their natal group or the group into which they were emigrating (Roper *et al.*, 2003). Moreover, observations made during the oestrous period described females mating with both resident males and a visiting male, who temporarily occupied a sett within the group territory. This arrangement provoked only minor rivalry and no major fights were seen (Paget & Middleton, 1974). Such results may indicate a high degree of relatedness both between and within groups (Cheeseman *et al.*, 1988, da Silva *et al.*, 1994, Woodroffe *et al.*, 1993). This was demonstrated in one high density population, where more than 80% of sexually mature offspring remained in their natal group (Cheeseman *et al.*, 1988, Woodroffe *et al.*, 1993). Mate guarding was less important in this population, most likely due to the high levels of intra-group relatedness (Woodroffe *et al.*, 1997). In these populations habitat saturation may prevent or delay individuals from dispersing permanently (Carpenter *et*

al., 2005, Woodroffe *et al.*, 1993). Instead males undergo forays into neighbouring groups to gain extra-group matings (Woodroffe *et al.*, 1997).

1.2 Reproduction

1.2.1 Reproductive Seasonality

Post mortem examinations spanning the past fifty years provide detailed descriptions of the reproductive cycle of the badger within its European range (Ahlund, 1980, Cresswell *et al.*, 1992, Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). These studies include gross pathology of the reproductive tract and in-depth histological analysis (Neal & Harrison, 1958), encompassing both low-density populations (Sweden, (Ahlund, 1980)) and one of the highest recorded (South-west England, (Cresswell *et al.*, 1992, Johnson & MacDonald, 2001, Johnson *et al.*, 2000)).

Cubs are born during the first three months of the year, January-March, with most parturitions occurring between late January and February in the south-west of England and Ireland (Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). There is a general trend towards earlier birth dates in the more southerly European populations, as was described for a population in the Donaña area of Spain, where most cubs were born in the first week of January (Revilla *et al.*, 1999). Litter size in both Ireland and the south-west of England averaged 3 foetuses (Whelan & Hayden, 1993 (mean =2.9); Neal & Harrison, 1958 (mean =3.1); Page *et al.*, 1994 (mean=2.9)). Lactation in reproductively successful females ceases in June/July, when cubs become fully independent (Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993) and sexual maturity is usually reached in the second year of life (12-24 months) for both sexes (Ahlund, 1980, Neal & Harrison, 1958, Whelan & Hayden, 1993).

Observational data from the south-west of England suggest that copulations can occur during any month between February and October (Neal & Harrison, 1958). Sexual excitement is greatest during February to May (Harrison & Neal, 1956) and spermatozoa

have been identified in cervical smears of a small number of females in February, March, June, August, October and December (Page *et al.*, 1994). Testicular weight, used as an indicator of sexual activity, increased rapidly to its greatest values during the early part of the year, at the onset of the breeding season, following which it declined gradually until November/December (Ahlund, 1980, Page *et al.*, 1994). Smears of the caput epididymis confirmed that a portion of adult male population showed active spermatogenesis throughout the year (Ahlund, 1980, Page *et al.*, 1994). In the south-west of England, frequencies of males with positive smears are high right through the year with the lowest frequencies occurring in October-December (Page *et al.*, 1994). In Sweden the decline in frequency happens earlier in the year from August onwards and involves greater reductions (Ahlund, 1980). The male pattern of activity can vary between and within populations (Mead & Wright, 1983). Periods of high testosterone secretion and spermatogenesis tend to coincide with the fertile period in females (Audy-Relexans, 1972, Mead & Wright, 1983, Woodroffe *et al.*, 1993). In some populations immigrant males appear to sustain spermatogenesis for longer periods than natal males, perhaps as a means to obtain most copulations. Conversely, in populations where dispersal is unlikely, males make forays into neighbouring groups. These males maintain spermatogenesis for a relatively short period of time; making the majority of males infertile while females are still sexually receptive (Woodroffe *et al.*, 1997).

Females first show signs of ovulation early in the year, during February-April, (Ahlund, 1980, Neal & Harrison, 1958, Paget & Middleton, 1974), with the peak in large pre-ovulatory follicles occurring in January-March (Cresswell *et al.*, 1992, Harrison & Neal, 1956). Ovulating females in February are composed of females in post-partum oestrus, primiparous females (first time breeders) and females who failed to reproduce during the previous breeding season (Paget & Middleton, 1974). From behavioural observations during the post-partum mating period the oestrous cycle has been estimated to have a length of 30-40 days (Paget & Middleton, 1974). Ovulations may occur throughout the year but there has been no definitive conclusion regarding the frequency of oestrous cycles or their timing. Neal and Harrison (1958) noted that ovulations may occur in June, September or October owing to the presence of follicular activity and vaginal cornification. Furthermore, ova were retrieved from a small number of females in

February, March, June, August and November (Page *et al.*, 1994). Cresswell *et al.* (1992) demonstrated that in addition to the peak in pre-ovulatory follicles during the post-partum period there was a second distinct peak in late summer/autumn, during July-September. The authors suggested that adult females had a series of oestrus cycles until they became pregnant and the second peak in late summer/autumn may constitute a single oestrus for a low number of adult female; this appeared to be typical in such high density populations. Alternatively, Harrison and Neal (1956) argued that females may ovulate for the first time either early in the year or during the summer, and possibly exhibit further ovulations during the breeding season.

The frequency of ovulation, that is, adult females with corpora lutea, increases rapidly during the early oestrus period and from May/June onwards it stays close to 100% (Ahlund, 1980, Page *et al.*, 1994). Ovulation rates were greater than fertilisation rates, suggesting that some females failed to become fertilised during their first, and possibly subsequent, oestrous cycles. It is possible that females undergo renewed ovulations until they conceive (Ahlund, 1980). Furthermore, corpus luteum number per sow increases during the year independent of blastocyst number (Ahlund, 1980, Cresswell *et al.*, 1992, Neal & Harrison, 1958, Page *et al.*, 1994). Females with additional corpora lutea represent those individuals that have no blastocysts owing to infertile matings (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Neal & Harrison, 1958), individuals that produced more ova than were fertilised (perhaps during more than oestrous cycle) (Ahlund, 1980, Cresswell *et al.*, 1992), and possibly individuals that have lost either all or a proportion of their blastocysts following fertilisation (Cresswell *et al.*, 1992, Harrison & Neal, 1956). Additional corpora lutea were especially common for those females with few blastocysts (Page *et al.*, 1994).

The presence of blastocysts indicates that fertilisation has occurred. Blastocysts were first observed in adult females at the end of March-April (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Page *et al.*, 1994, Whelan & Hayden, 1993). Maximum fertilisation frequencies varied between studies. Frequency peaked from June onwards (90-95%) in Sweden and this proportion was maintained during implantation and through

into gestation (Ahlund, 1980). Studies in Ireland and the south-west of England found that maximum fertilisation frequencies were attained either early in the year (Cresswell *et al.*, 1992 (March, ~80%)) or later in autumn (Whelan & Hayden, 1993 (September, 80-90%); Page *et al.*, 1994 (August, 94%)), but there was a slight decrease in this proportion prior to implantation in all cases. This suggests that a proportion of females within these populations lost their blastocysts prior to implantation. Moreover, mean blastocyst number, which was approximately three in both Ireland and the south-west of England (Neal and Harrison, 1958 (mean=2.92, range=1-5); Cresswell *et al.*, 1992 (mean =3.7); Whelan & Hayden, 1993 (mean=2.7, range=1-5); Page *et al.*, 1994 (mean=2.78, range=1-6)), was perceived to decrease during the breeding season in one population in the south-west of England (Cresswell *et al.*, 1992). This form of reproductive failure may not occur in Swedish populations, with all fertilised females going on to implant all available blastocysts (Ahlund, 1980).

Following fertilisation, females enter a period of diapause, where the blastocyst is essentially in a state of quiescence and implantation is delayed for up to 10 months (Perry, 1971) (this mechanism will be discussed in further detail in Section 1.2.2). However, blastocyst diameter does increase during the period of diapause (Ahlund, 1980, Cresswell *et al.*, 1992, Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). Substantial increases to blastocyst diameter are achieved prior to implantation in November-January (Ahlund, 1980, Neal & Harrison, 1958, Whelan & Hayden, 1993). Therefore, it is possible to determine approximate fertilisation times from measurements of blastocyst diameter. As previously mentioned, corpora lutea number increases through the year independent of blastocyst number, suggesting that further ovulations have taken place, even late on during the period of diapause (Harrison & Neal, 1956, Neal & Harrison, 1958). Moreover, blastocycts with diameters indicative of recent fertilisations were observed in Ireland and the south-west of England during the second half of the year, corresponding with an increase in the proportion of females with blastocysts (Cresswell *et al.*, 1992, Page *et al.*, 1994, Whelan & Hayden, 1993).

Females in this condition belonged to one of three categories: yearlings that had corresponding numbers of corpora lutea to blastocysts, suggestive of first time breeders

reaching sexual maturity late in the year (Cresswell *et al.*, 1992); adults that had an excess of corpora lutea, possibly owing to unsuccessful fertilisation or loss of blastocysts (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Neal & Harrison, 1958); or adults that had two distinct sizes of blastocyst and a corresponding number of corpora lutea (Ahlund, 1980, Cresswell *et al.*, 1992). The latter group provide evidence of possible superfetation, that is, ovulating and becoming fertilised while already pregnant (Cresswell *et al.*, 1992). Observation of cyclic changes to the vaginal epithelium (Neal & Harrison, 1958) and evidence of folliculogenesis (Harrison & Neal, 1956), while healthy blastocysts are present in the uterus, as well as ova being retrieved from successfully fertilised females (Harrison & Neal, 1956, Page *et al.*, 1994) provides further support for the possibility of superfetation. Moreover, Page *et al.* (1994) noted that in July-October the number of corpora lutea continued to increase while the frequency of fertilised females remained the same, implying that further ovulations occurred in sows that were already pregnant.

Superfetation may not occur in all populations. Ahlund (1980) found only one example within the Swedish sample and suggested that superfetation may be a rare event. However, sampling was low towards the end of the year so examples may have been missed. Conversely, the frequency of superfetation in populations in Ireland and south-west England sampled during September to December seem quite substantial at 14% and 21%, respectively (Cresswell *et al.*, 1992, Whelan & Hayden, 1993). Superfetation provides females with additional opportunities to become fertilised and to mate with multiple males, resulting in mixed paternity litters. Although mixed paternity is not necessarily indicative of superfetation, with females capable of mating with multiple males during a single oestrus, it has been reported that 16% of litters (N=31) tested during a genetic study in south west England had mixed paternity (Carpenter *et al.*, 2005).

1.2.2 Delayed Implantation

In some species, the blastocysts can remain free in the uterine lumen, not attaching to the uterine wall, for a prolonged period of time, leading to a corresponding extension in gestation time (Sandell, 1990). During this period the blastocyst essentially enters a state

of suspended animation, metabolic activity is reduced, growth is very slow, and cell division and expansion is limited (Hogarth, 1978, Renfree & Calaby, 1981, Renfree & Shaw, 2000). This phenomenon is known as delayed implantation and was first described by German anatomist Zeigler when he witnessed a blastocyst in diapause in the roe deer (*Capreolus capreolus*) in 1841 (Aitken, 1981). Delayed implantation can be classified as either lactational (facultative) or seasonal (obligate) (Hogarth, 1978, Sandell, 1990). Seasonal delayed implantation has been described in 47 mammalian species in ten families, and may have evolved independently at least 17 times (Sandell, 1990). Delayed implantation is common within the mustelid family, with 17 species showing embryonic diapause, e.g. badgers, western spotted skunk, marten, and wolverine (Hogarth, 1978, Mead, 1981, Sandell, 1990). With seasonal delayed implantation, activation of the diapausing embryo occurs at a particular time of the year for all females within the population. The delay tends to be long and gestation is extended over a greater part of the year enabling both mating and birth to occur at suitable times (Hogarth, 1978, Sandell, 1990). In the Eurasian badger, the diapausing blastocyst can remain free within the uterine lumen for up to 10 months (Perry, 1971), with activation and implantation occurring during December/January for the majority of females (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981).

Progesterone secreted by the corpus luteum is required for growth and development of the blastocyst (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981, Martinet *et al.*, 1981). Therefore, reduced corpus luteum activity is essential for maintenance and control of delayed implantation (Renfree & Calaby, 1981). Seasonal delayed implantation in mustelids is believed to result from insufficient secretion of gonadotropins by the pituitary (Hogarth, 1978, Mead, 1993). This leads to incomplete differentiation of the luteal cells within the corpus luteum, making it inactive and thereby reducing progesterone secretion (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981, Mead, 1981, 1993, Renfree & Calaby, 1981). Consequentially, the uterus fails to undergo sufficient development to facilitate continuous embryonic development (Hogarth, 1978, Mead, 1981, 1993, Renfree & Shaw, 2000). Nevertheless, the low levels of progesterone secreted during the period of delayed implantation (Bonnin *et al.*, 1978) are sufficient to maintain blastocyst viability; as was demonstrated by an experimental ovariectomy which

resulted in death of the diapausing blastocysts (Mead, 1993). Oestrogens may be indirectly involved in blastocyst maintenance, possibly being responsible for inducing and maintaining progesterone receptors within the uterus (Canivenc & Bonnin, 1981, Mead, 1993, Mondain-Monval *et al.*, 1980). It is the accumulation of fluid within the blastocoel which causes the gradual growth of the blastocyst observed throughout the period of diapause (Neal & Harrison, 1958).

Resumption of full ovarian activity, especially of the corpus luteum, is initiated by one or more gonadotropins, which are produced by the anterior pituitary prior to implantation (Mead, 1981, 1993). These pituitary hormones are essential for complete luteal cell differentiation; hypophysectomy (removal of the pituitary gland) prior to full luteal activity was reported to result in failed implantations (Mead, 1981, 1993). The fully differentiated luteal cells secrete increased levels of progesterone which induce alterations to the uterine environment. Consequently, the environment within the uterus is capable of facilitating renewed embryonic development resulting in rapid growth and implantation of the blastocyst (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981, Mead, 1981, 1993, Perry, 1971). The process of implantation involves a number of synchronised steps; neither the administration of progesterone and/or oestrogens alone were capable of stimulating implantation (Canivenc & Bonnin, 1981, Mead, 1981, 1993, Perry, 1971).

Light acting via the eyes appears to stimulate the hypothalamic-pituitary axis, resulting in increased secretion of essential gonadotropins and hence renewed luteal development, which are the first steps in the implantation process (Mead, 1981, 1993). In the northern hemisphere maximal night-time hours occur in December, which is also when implantation happens. It is most likely that it is this reduction in photoperiod that triggers the implantation process (Canivenc & Bonnin, 1981, Mead, 1981, 1993). Wild female badgers in delayed implantation were observed under conditions of 'artificial winter' (+5°C in short photoperiod). It was reported that the females gradually adapted their behaviour and ovarian function to these new experimental conditions. After time, there was a sharp increase in progesterone, renewed embryonic development, implantation and successful parturition. Implantation occurred in July, six months earlier than animals

living in the wild. Besides the alteration in timing, implantation proceeded as was expected under natural circumstances (Canivenc & Bonnin, 1979, Canivenc & Bonnin, 1981). Nutritional status prior to implantation may also influence implantation date, with females of relatively high body condition implanting earlier in the season. Females may use body condition as an indicator of their own local conditions, altering the timing of implantation accordingly (Woodroffe, 1995).

Pressure to protect reproduction is one of the fundamental influences of evolution (Renfree & Shaw, 2000). The majority of authors have agreed that delayed implantation is an adaptation that allows mating and parturition to occur at the most favourable times of the year by effectively lengthening the gestation period (Renfree & Shaw, 2000, Sandell, 1990). Delayed implantation is likely to increase female fitness and has been reported to have evolved independently at least 17 times (Renfree & Shaw, 2000, Sandell, 1990). The great adaptive advantages that delayed implantation provided in some species may relate to former ecological conditions and may now be considered limited. Once established this adaptation is most likely retained because it is associated with minor costs; being neutral and/or having no negative selective characteristics (Mead, 1993, Sandell, 1990). It is possible that such a mechanism may have occurred within the badger species leading to the retention of delayed implantation.

In order to maximise reproductive potential mating should occur when individuals are in prime condition and when the opportunity for female choice and male competition is greatest. At the same time, parturition should be timed to enable rearing to coincide with the period of greatest resource availability (Sandell, 1990). Mate choice theory suggests that females will select the best available male to mate with, thereby increasing their fitness. Therefore, females should come into oestrus when the possibilities of choice are greatest and when they can create the greatest levels of competition between possible mates (Sandell, 1990). Female badgers may visit boundary latrines to advertise their reproductive status, possibly creating aggression between competing males and enabling greater opportunities for female choice to occur (Stewart *et al.*, 2002). The best time for males to compete for mating opportunities is when high quality resources are readily available, enabling them to be in prime condition (Sandell, 1990). Badgers tend to live in

inter-sexual groups within a fixed territorial range; in this situation male competition is spread throughout the year, which allows for a prolonged mating period (Sandell, 1990).

Parturition in badgers takes place in February and March, a time when high quality food availability is increasing, thus minimising the costly reduction in body condition that happens during lactation (Woodroffe & MacDonald, 1995a). In addition, cubs can take advantage of the high levels of food availability during the weaning period, thereby maximing survival rates (Woodroffe, 1995). Those females which implant earlier, owing to better body conditions (Woodroffe, 1995), may have cubs which gain earlier independence (Sandell, 1990). This may influence the cubs, by further increasing their chances of survival, and also provides the female with more time to recover during the favourable season (Sandell, 1990).

1.2.3 Male Reproductive Hormones

Although male badgers display active spermatogenesis throughout the year, they are still considered seasonal breeders, as testicular activity tends to fluctuate between seasons season (Ahlund, 1980, Audy *et al.*, 1985, Audy, 1976, Page *et al.*, 1994). Testicular activity, determined by increases in testicular weight (Page *et al.*, 1994), recommences in autumn and is followed by a reproductive season beginning in winter and continuing until summer (Maurel *et al.*, 1984). Concurrently, testosterone fluctuates at its highest levels from December to June with significantly lower levels occurring between July and November (Audy *et al.*, 1985). Therefore, endocrine-testicular-activity is said to begin in January or February, and continues for a number of months until July (Maurel *et al.*, 1984). More recent studies have displayed a more protracted period of endocrine-testicular-activity, beginning early in the year and gradually declining towards late summer/autumn (Ahlund, 1980, Page *et al.*, 1994, Woodroffe *et al.*, 1997)

Activation of the hypophyso-gonadal axis occurs in Autumn prior to the winter solstice (Maurel *et al.*, 1984), when the day-light photoperiod is decreasing and the light period is shorter than the period of darkness (Audy *et al.*, 1985, Maurel *et al.*, 1984). The reduction in daylight hours, during the period of minimal testicular activity may be responsible for

triggering pituitary activity, which stimulates the episodic release of lutenising hormones. This leads to an increase in testosterone levels and, hence, testicular activity at the onset of the reproductive season in winter (Audy *et al.*, 1985).

Testosterone is released in pulses (Maurel *et al.*, 1981), with the highest frequency and amplitude of pulses occurring during breeding (active testicular phase) and to lesser extent post-breeding (regressing testicular phase) (Audy *et al.*, 1985). However, baseline testosterone levels may not be a reliable indicator of fertility, as males often have an excess of testosterone, which is much higher than those levels needed to maintain spermatogenesis (Wingfield *et al.*, 1990, Woodroffe *et al.*, 1997)

1.2.4 Female Reproductive Hormones

Plasma progesterone levels secreted by the corpora lutea correlate closely with events in the reproductive cycle (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981). The period of delayed implantation can last for up to ten months beginning with fertilisation in February/March and continuing until implantation in December/January (Perry, 1971). Low levels of progesterone (Bonnin *et al.*, 1978), resulting from a lack of luteal activity in the corpora lutea are essential for maintaining delayed implantation (Canivenc & Bonnin, 1981, Mead, 1981, 1993, Renfree & Calaby, 1981). Progesterone levels follow a bi-modal pattern of release during the year: the first and lesser peak during July to September (Bonnin *et al.*, 1978) originates from the resumption of low levels of luteal activity which stimulates secretions within the uterine endometrium (Bonnin, 1964). The second peak occurs during December to February and is associated with full luteal activity, implantation and gestation (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981, Mead, 1981, 1993, Mondain-Monval *et al.*, 1980, Perry, 1971). Although the corpora lutea persist until the end of gestation, they show signs of involution mid-pregnancy (Harrison & Neal, 1956). The placenta is thought to be involved in progesterone secretion and undergoes rapid development between days 15-30 of the gestation period, most likely in conjunction with the decline in the activity of the corpora lutea (Mead, 1993).

The reduction in daylight photoperiod may be responsible for the resumption of pituitary activity in October. This stimulates the release of gonadotrpins prior to the increase in progesterone levels and implantation, which occurs several months later in December (Canivenc & Bonnin, 1981).

Oestrogen levels fluctuate during the period of delayed implantation, being highest during May to October (Mondain-Monval *et al.*, 1980). Concurrently, during this period of heightened oestrogen secretion, activity is observed both in the ovary and the vaginal epithelium: folliculogenesis is seen in the ovary with many follicles reaching advanced stages of maturity; proliferation of the vaginal epithelium occurs several times during May to September; and keratinisation of the epithelium is observed in May, June and September (Mondain-Monval *et al.*, 1980).

1.2.5 Reproductive Failure

Density may have a considerable effect on the reproductive potential of a population. In Sweden, which is considered a low density population, 90-95% of the adult population were fertilised and an equivalent proportion went on to implant (Ahlund, 1980). Post natal losses were not assessed, but such fertilisation and implantation rates far exceed those of other studies (Cresswell *et al.*, 1992, Whelan & Hayden, 1993). Similarly, 65% of females were reported to breed successfully in the Donaña area of Spain, thought to be one of the lowest densities ever recorded (Revilla *et al.*, 1999).

In Ireland and the south-west of England, there appears to be a considerable reserve in reproductive capacity for both populations (Cresswell *et al.*, 1992, Page *et al.*, 1994, Whelan & Hayden, 1993). Only 30-40% of the reproductive potential of the population was achieved (Whelan & Hayden, 1993: (35-40%), Cresswell *et al.*, 1992: (30%)); with reproductive failure occurring at all stages of the reproductive cycle (Cresswell *et al.*, 1992). The biggest losses seem to occur during the implantation process for populations in the south-west of England (Cresswell *et al.*, 1992, Page *et al.*, 1994). Blastocysts were lost at implantation and females seemed to employ an all-or-nothing strategy of implantation (Page *et al.*, 1994, Whelan & Hayden, 1993). In Ireland foetal/cub mortality

was more influential in the reduction in reproductive potential as implantation frequencies were nearly twice as great as lactation frequencies (Whelan & Hayden, 1993).

Not all females successfully breed in consecutive years (Neal & Harrison, 1958). The probability of breeding over successive years was estimated at 0.8 for an Irish study population, which may have been an overestimate due to the methodology used (Whelan & Hayden, 1993). Age may have a significant effect on breeding success. Younger females were found to be less successful in breeding, having lower fertilisation rates (Ahlund, 1980, Page *et al.*, 1994, Whelan & Hayden, 1993), lower blastocyst numbers (Page *et al.*, 1994, Whelan & Hayden, 1993), greater reductions in the proportion of females with blastocysts pre-implantation (Whelan & Hayden, 1993), and lower pregnancy rates (Ahlund, 1980). Cresswell *et al.* (1992) found that few females bred before their fourth year of life, and a study using genetics to assign parentage found that few individuals aged less than two years or greater than eight years were successful breeders (Carpenter *et al.*, 2005).

1.2.6 Density-Dependent Constraints

Density dependent constraints on fecundity and cub survival are responsible for the regulation of population stability and tend to function at densities close to the carrying capacity (Anderson & Trewhella, 1985, Tuyttens *et al.*, 2000b). The number of reproductively active females increases with territory quality and hence food abundance (Tuyttens *et al.*, 2000b, Woodroffe & MacDonald, 1995b). However, as density reaches carrying capacity, which may be low in some poor quality territories, food becomes a limiting factor and fecundity decreases with increasing group size (da Silva *et al.*, 1993, MacDonald *et al.*, 2002a, Tuyttens *et al.*, 2000b). Therefore, density dependent constraints on fecundity and cub survival are an indirect response to limited resources in populations which are approaching carrying capacity. Limited resources may directly affect reproductive success by reducing body condition. Alternatively a reduction in resource availability may increase breeding competition and reproductive suppression which in turn will reduce reproductive success. Within some populations only a single, possibly dominant, female may breed successfully in a social group (Kruuk, 1978, Revilla

et al., 1999) whereas multiple females are reported to breed in other populations (Carpenter *et al.*, 2005, Domingo-Roura *et al.*, 2003, Woodroffe & MacDonald, 1995b).

Body condition can be an influential factor in reproductive success. High fat reserves are necessary to counteract the reduction in body condition which occurs during lactation. Females in relatively poor condition in autumn, prior to breeding, were more likely to fail in subsequent gestations, producing no cubs in the spring. This may be a control against potential mortality owing to the costly period of lactation and/or a response to low food availability (Delahay *et al.*, 2006a, Woodroffe & MacDonald, 1995a, Woodroffe & MacDonald, 1995b) Close to carrying capacity, food availability may become limited, creating a negative relationship between an individual's body weight, body condition and group size (MacDonald *et al.*, 2002a, Rogers *et al.*, 1997). This results in a reduction in the number of actively breeding females (Rogers *et al.*, 1997) and fecundity rates decline (MacDonald *et al.*, 2002a). Therefore, the reduction in body weight and condition may be associated with a drop in reproductive success. Furthermore, during periods of extreme food decline in areas otherwise associated with high resource abundance (Woodroffe & MacDonald, 1995b), competition may shift, in larger groups, from that of gaining breeding status to feeding competition. Individual body condition levels may be more important in determining reproductive success than social status during these extreme circumstances (Woodroffe & MacDonald, 1995b).

Female competition for breeding status can result in active reproductive suppression of subordinates. Sows have been known to interfere directly with mating attempts in captivity (Kruuk, 1989), prevent implantation by aggression (Cresswell *et al.*, 1992), and possibly carry out infanticide (Cresswell *et al.*, 1992, Kruuk, 1989, Woodroffe & MacDonald, 2000), although this may have simply been a case of cannibalism following death of the cubs. Such extreme reproductive suppression may be a response to limited resources leading to the regulation of a sustainable group size (Woodroffe & MacDonald, 1995b). Only a single female was reported to reproduce in an area of low food availability in Scotland. It was speculated that within this low density population that it was only the

dominant female that would reproduce and that the subordinates within the group were actively prevented from breeding (Kruuk, 1989). Similarly, only one female was ever reported to breed in groups from a low density population located in south-western Spain, an area where food abundance is strongly seasonal (Revilla *et al.*, 1999, Revilla & Palomares, 2002). Although no evidence of direct reproductive suppression has been reported for this population, the reproductive female used all the available territory during the season of lowest food availability and overall had a higher body condition index (Revilla & Palomares, 2002). Therefore, possibly by excluding subordinates during periods of low food availability, dominant females may ensure a sufficient increase in body condition to facilitate reproduction and simultaneously restrict the consequences of low food availability onto subordinates, resulting in a reduction in their reproductive success.

In southern England, although multiple females are reported to breed successfully (Carpenter *et al.*, 2005, Domingo-Roura *et al.*, 2003, Woodroffe & MacDonald, 1995b), social status is still influential in reproductive success. During one study, population density was determined to be high but was not approaching carrying capacity (Woodroffe & MacDonald, 1995b). Density-dependent constraint was not a factor affecting breeding competition in this population as resource abundance was plentiful. Although almost all mature females were of suitable condition to reproduce successfully, it was only the larger females, who held more exclusive ranges, that went on to produce cubs, indicating reproductive suppression by these more dominant individuals (Woodroffe & MacDonald, 1995b).

1.2.7 Avoidance of Reproductive Suppression

Females may avoid reproductive suppression and aggression by moving to a nearby sett (outlier or annexe sett) which is not part of the main sett (Cresswell *et al.*, 1992, Stewart *et al.*, 1999). The presence of an annexe sett correlated with an increased productivity in younger females (Cresswell *et al.*, 1992). Moreover, habitat which became available as a consequence of badger removal operations was recolonised by young females from

adjacent groups, who had not previously reproduced. This further supports the theory that, given the opportunity, 'subordinates' will move to areas away from the main sett in an attempt to avoid reproductive suppression and increase breeding opportunities (Tuyttens *et al.*, 2000b).

Anecdotal evidence suggests that those females who remain in the main sett complex during the late stages of gestation and parturition may move from communal sleeping chambers to one that they can monopolise (Neal & Cheeseman, 1996). It has also been reported anecdotally that females tend to live separately from the males during this period (Paget & Middleton, 1974). Both of these behaviours are possibly attempts to avoid antagonism towards themselves and their young (Kruuk, 1989). Females may benefit from actively enlarging the sett complex by creating additional chambers in which to avoid direct competition (Stewart *et al.*, 1999). Reproductive females who were closely associated with the sett (highly resident), were more likely to spend time digging than transient individuals or those that spent less time at the sett (Stewart *et al.*, 1999). Furthermore, highly resident males, considered to be of high reproductive status, were also observed to partake in digging more often than those of low association and status. This, too, may be an attempt to increase survivorship of their sired young (Stewart *et al.*, 1999).

An alternative incentive for using outlier setts or separate chambers of the main sett may be ectoparasite avoidance. Individuals that retreated to outlier setts tended to be younger and had higher flea burdens; using these setts as a place to recover from ectoparasite infestations (Roper *et al.*, 2001). In this study, occupancy of outlier setts did increase from March, which is associated with parturition, but it never exceeded more than 21 days, therefore it is unlikely that young were being raised in these setts (Roper *et al.*, 2001). Moreover, individuals rarely occupied sleeping chambers exclusively; during the summer and autumn period individuals spread out more within the main sett complex but they usually remained in pairs or trios (Roper *et al.*, 2001).

1.3 Summary of Study

1.3.1 Background

Ireland has been involved in a national bovine tuberculosis eradication scheme since 1954 (Griffin *et al.*, 2003). Prevalence of *Mycobacterium bovis* infection initially declined during the first decade of the scheme, but low incidence levels have persisted among the national herd. In the 1970's, *Mycobacterium bovis* infection was identified in the badger and it became apparent that badgers may provide a possible wildlife reservoir for bovine tuberculosis in both Ireland and the UK (Muirhead *et al.*, 1974, Noonan *et al.*, 1975). Subsequently much work has been done to provide a possible link between the disease in this wildlife species and that in domestic cattle. In Ireland, positive results have been obtained from two studies, the East Offaly Project (Eves, 1999) and the Four Area Study (Griffin *et al.*, 2003), which assessed the effectiveness of reactive culling at reducing bovine tuberculosis in cattle. In light of these findings the Department of Agriculture, Fisheries and Food (DAFF) have adopted a controlled nationwide scheme of reactive badger removal (O'Mairtin *et al.*, 1998), which has been more consistent since 2002-2003.

There has been much controversy regarding the effectiveness of badger culling as a control for bovine tuberculosis in cattle. Although positive results have been achieved in Ireland (EOP: (Eves, 1999); Four Area Study: (Griffin *et al.*, 2003) studies in the UK have shown an increased disease incidence in cattle in areas where culling has previously occurred (Donnelly *et al.*, 2003, Vicente *et al.*, 2007, Woodroffe *et al.*, 2006). Possible perturbation effects following periods of reactive culling have been suggested as a reason for this increase. The marked discrepancy between studies conducted in Ireland and those in the UK may be due to variations in population dynamics and/or trapping methodologies. Therefore, it became apparent that there may also be discrepancies in the basic ecology of these two populations. The control programme provided an excellent opportunity to conduct a detailed analysis of the reproductive biology of the badger in Ireland.

The ecology of the Eurasian Badger may be highly diverse; varying greatly between geographical regions, populations and even adjoining groups. In the past, there have been a disproportionate number of studies conducted in UK; this has created a considerable bias in the literature (Neal & Cheeseman, 1996). Moreover, group sizes in southern England, where the majority of studies have taken place, are uncharacteristically large for this species. This coupled with occupancy of very small territories, due to super-abundance of resources, has led to some of the highest densities reported within the badger's range (Cresswell *et al.*, 1992, Johnson & MacDonald, 2001, Johnson *et al.*, 2000). In contrast, densities within the Irish population have been described as being low to medium (Feore & Montgomery, 1999, Sleeman *et al.*, 2009). It has been argued that mating systems result from individual strategies rather than being an evolved feature of each species (Clutton-Brock, 1989). Therefore, despite close geographical proximity of populations within Europe, such variations in population dynamics may greatly influence the reproductive strategy and potential of the populations. Therefore, this study provided an unparalleled opportunity to detail the reproductive seasonality of the male and female badger in a low density population, as a comparison to previous studies from the south-west of England and parts of Europe.

Furthermore, it has been 50 years since the last extensive histological study of reproductive seasonality in the badger (Southern England, N=70 (Neal & Harrison, 1958)). This study provides an in-depth histological analysis, utilising a far greater sample size (N=580) and encompassing all months of the year. It is the combination of morphological, histological and hormonal data from both sexes that makes this study the most thorough to have been undertaken so far, presenting a uniquely comprehensive picture of reproductive seasonality in the badger

1.3.2 Aims & Objectives

- To describe the basic demography of the study population
- To describe in full the reproductive seasonality of the male and female badger
- To relate the reproductive endocrine cycle to reproductive events
- To assess the reproductive strategies and potential of the population

- To compare and contrast the reproductive strategies of males and females within the Irish population

2 MATERIALS AND METHODS

2.1 General Tissue Collection

2.1.1 Study Population

The project used a sample of the badgers that were removed from the population as part of the nationwide control programme for the eradication of Bovine Tuberculosis (BTB) in the Republic of Ireland. These badgers were sent to the Irish Equine Centre (IEC), where a routine post mortem examination was conducted by Dr. Ursuala Fogarty for the presence of BTB. This allowed the remainder of the carcass to be utilised for reproductive and demographic analysis. Badgers were examined between March 2005 and March 2006 and between June and September 2006. There were few badgers available for examination during the summer/autumn period of 2005 (June-September); therefore, badgers were examined in the latter period to supplement the sample size for that time of the year.

Badgers were taken from the northern region (counties Donegal, Cavan and Monaghan) (Figure 2-1) and southern region (counties Cork and Clare) of the country. During the summer/autumn periods, June to September 2005 and 2006, badgers were also taken from 15 additional counties to increase the sample size. Badgers from September to June 2006 were examined at University College Dublin as part of separate study and included road casualties.

The additional counties were divided into Northern and Southern regions, based on how the district veterinary offices for these counties are categorised by the Department of Agriculture Fisheries and Food (DAFF). Sligo, Mayo, Cavan, Galway, Longford, Meath, Dublin and Offaly were considered to be North of the country while Kildare, Wicklow, Tipperary, Kilkenny, Carlow, Kerry and Waterford were considered South (Figure 2-1).

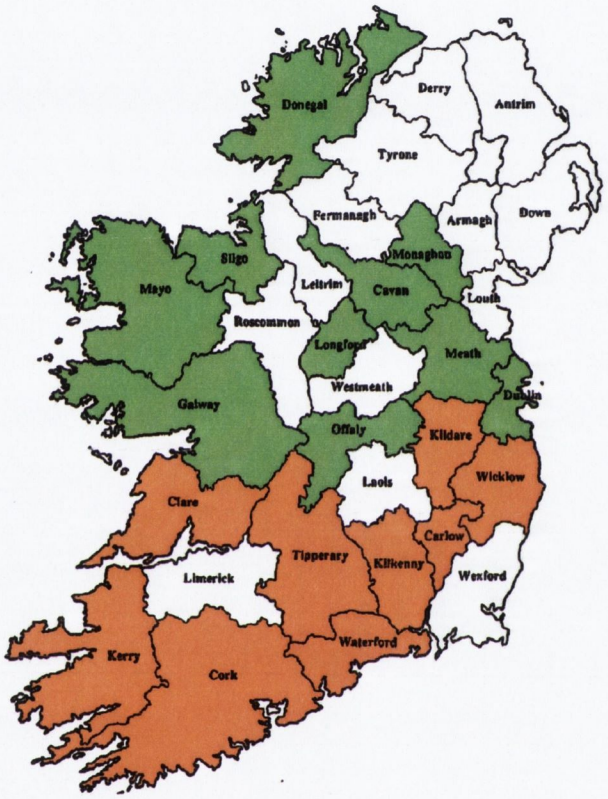


Figure 2-1: Map of Ireland showing study areas divided into Northern and Southern regions. Counties shaded in ■ represent the Northern region and counties in ■ represent the Southern region; no badgers were received from the un-shaded counties.

2.1.2 Post Mortem Examination Procedure

The date, badger number, county of origin, sett of origin (sett number), body weight (nearest 0.1kg) and sex of each badger were recorded. All the specimens collected were labelled with badger number, month and tissue.

The following morphological measurements were recorded: nose-tip to base-of-tail length (cranial end of the first coccygeal vertebra) (mm), and heel to toe length-of-hind-foot (mm). Bite wounds occurring on the head/neck, legs, rump/tail and elsewhere were recorded. Severity of the wounding and healing was scored on a scale of 1-6 as previously used by Cresswell *et al.* (1992). Subcutaneous fat depth (nearest 0.5mm), as a measure of body condition, was measured by making an incision in the mid-lumbar region and measuring the depth of the fat perpendicular to the skin surface using a ruler. Mammary glands were assessed visually for evidence of lactation, and the second last gland was collected. Hair from the surface of the mammary gland and any excess tissue was removed. The mammary gland was stored in 10% formalin for histological processing.

The complete reproductive tract of the female, including the ovaries, uterus, cervix and vagina, was collected. No special precautions were taken to prevent loss from the uterus as the cervix creates a natural seal. Prior to the removal of the uterus, a small incision was made in the fat surrounding the left ovary to provide a means of identification.

In the males, the testes including the epididymis, seminal vesicle, and baculum were collected. Each testis was stored separately.

The mandible and femur were collected. The mandible was stored in 10% formalin. The soft tissue (muscle, fat and fascia) was removed from the femur and the length of the femur from the head to the base, at the longest point, was measured using electronic calliper (nearest 0.01mm). The right kidney and its associated perirenal fat were dissected from the abdominal cavity. The kidney, excluding the surrounding perirenal fat, and the removed fat, were weighed separately (nearest 0.01g).

A blood sample was taken from the heart and thorax with the maximum possible volume being collected, this generally varied between 5 to 25 ml.

2.2 Examination of the Female Reproductive Tract

2.2.1 Gross Examination

The uterus was flushed to remove any unimplanted blastocysts. Blastocysts are the first visible stage of embryonic development and are located free within the uterine lumen throughout the period of diapause (Sandell, 1990). To flush the uterus the reproductive tract was laid out, any twists in the uterine horns were removed, so that the tract was completely flat with no obstructions. The length of each uterine horn, from the distal (ovarian) end to where the junction is formed at the uterine body was measured to the nearest 0.01mm.

A section of the vagina was removed to reveal the cervix. The opening of the cervix was located and a catheter inserted. A syringe containing 10ml of saline was attached to the catheter and a small amount of fluid was injected into the uterine horns to expand them. A section of fat was removed and wrapped around the base of one of the uterine horns. A forceps was then clamped over the fat to seal the horn. More saline was pumped into the uterine horn so that it was fully expanded and a small incision was made into the lumen close to the ovary using a scissors (Figure 2-2 (a)). Approximately 10 to 20ml of saline was flushed through the horn and the expelled fluid was collected. The forceps were removed and the flushed horn was then clamped. The flushing procedure was repeated for the second horn. The fluid from each horn was transferred into separate screw-top jars.

The fluid collected from the uterine horns was transferred into a gridded petri dish and scanned for the presence of blastocysts at ×125 magnification using a dissecting microscope (Make & Model: Olympus Szx9) (Figure 2-2 (c)). The diameter of each blastocyst was measured at the widest point using a graticule attached to the eye-piece of the microscope (1unit = 0.08mm at ×125 magnification) (Figure 2-2 (b)). These were then transferred into separate 1ml vials.

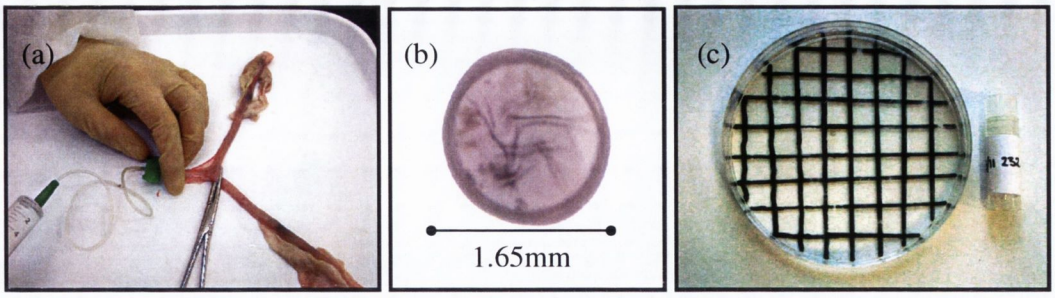


Figure 2-2: (a) Flushing of the uterus; (b) Diameter of blastocyt measured at widest point (×125); (c) Gridded Petri dish containing fluid from uterine horn.

The ovaries were removed and the diameter (nearest 0.01mm) and weight (nearest 0.01g) were measured. They were then stored separately in 10% formalin for histological processing. A transverse section, 1mm wide, was taken from the top of each uterine horn and a section of the vagina was removed just above the cervix. These were also stored in 10% formalin for histological processing.

An incision was made down the length of each uterine horn from the distal end to where the junction is formed at the uterine body. The internal surface of the uterine wall, the endometrium, was visually examined with the naked eye for placental scars and blastocysts. Placental scars were recognised on the endometrium as uniform bands of black colouration. Grey bands, which were more heterogenous in colour, were also observed. These grey placental scars were assumed to be older than those which were darker and more uniform in colouration. Therefore, the black placental scars were recorded as 'recent' and the grey 'faded'. The number and position of the placental scars was recorded. No blastocysts were located during the process of examining the endometrium. This suggests that uterine flushing is an efficient method for retrieving all available blastocysts.

During January and February, the season when badger sows were pregnant, it was not always possible to flush the uterus. When this was the case, an incision was made down the length of each uterine horn, and the fluid from within the horns was collected. The foetuses were removed from their surrounding embryonic sac and placenta. Each horn was further rinsed with saline and this was added to the fluid previously collected. The

positions of the foetuses within the uterus and the length of each foetus (crown of head-to-rump) were measured using electronic calliper (nearest 0.01mm). The weight of each foetus and any observed abnormalities were recorded (nearest 0.01g). During January and February females were classified as not pregnant (foetuses absent), pregnant (foetuses present) or post partum (foetuses absent but evidence of recent pregnancy: dark placental scars and increased length and diameter of uterine horns).

The weights of the foetuses were used to calculate implantation date in accordance with the formula described by Huggett and Widdas (Huggett & Widdas, 1951):

$$t_0 = t_g - \frac{\sqrt[3]{W}}{a}$$

Where t_0 is the number of days till parturition,

t_g is gestation length taken as 52 day (gestation length was described as 49-56 days by (Neal & Harrison, 1958)

W is average foetal weight,

and a is the growth velocity of 0.1295 (calculated for badgers by (Frazer & Huggett, 1974).

2.2.2 Histological Processing

Each ovary was cut longitudinally into roughly three 1mm thick sections and examined at ×63 magnification using a dissecting microscope. The colour of the ovary and the number, position, colour and diameter (using a graticule: 1 unit = 0.16mm at ×63 magnification) of any corpora lutea or follicles were recorded. A transverse section was taken from each sample of the uterine horn. The vagina was cut transversely obtaining a number of 1mm sections, including a section through the cervix. The mammary gland was cut at right angles to the skin, through the nipple: one or two sections approximately 1mm thick were taken depending on the diameter of the nipple. The sections of each tissue were transferred into four separate cassettes as follows: the section of the left ovary and uterine horn; the section of the right ovary and uterine horn; the sections of the

vagina; and the sections of the mammary gland. The tissue was processed as described in Appendix I.

2.2.3 Histological Examination of the Reproductive Tract

The oestrous cycle involves four stages, pro-oestrus, oestrus, metoestrus, and dioestrus, which are associated with physiological and cellular changes of the reproductive tract. Females who do not exhibit regular oestrous cycles are classified as anoestrous (Senger, 1997). The endometrium of the uterus and the epithelium of the vagina were examined at ×400 magnification (Make & Model: Olympus Bx41) and females were assigned to one of the stages of the oestrous cycle or were considered to be in a state of anoetrous.

Table 2-1: Histological description of the uterine endometrium used for identification of stages in the oestrus cycle; adapted from (Bacha & Bacha, 2000) and (Dellmann & Eurell, 1998).

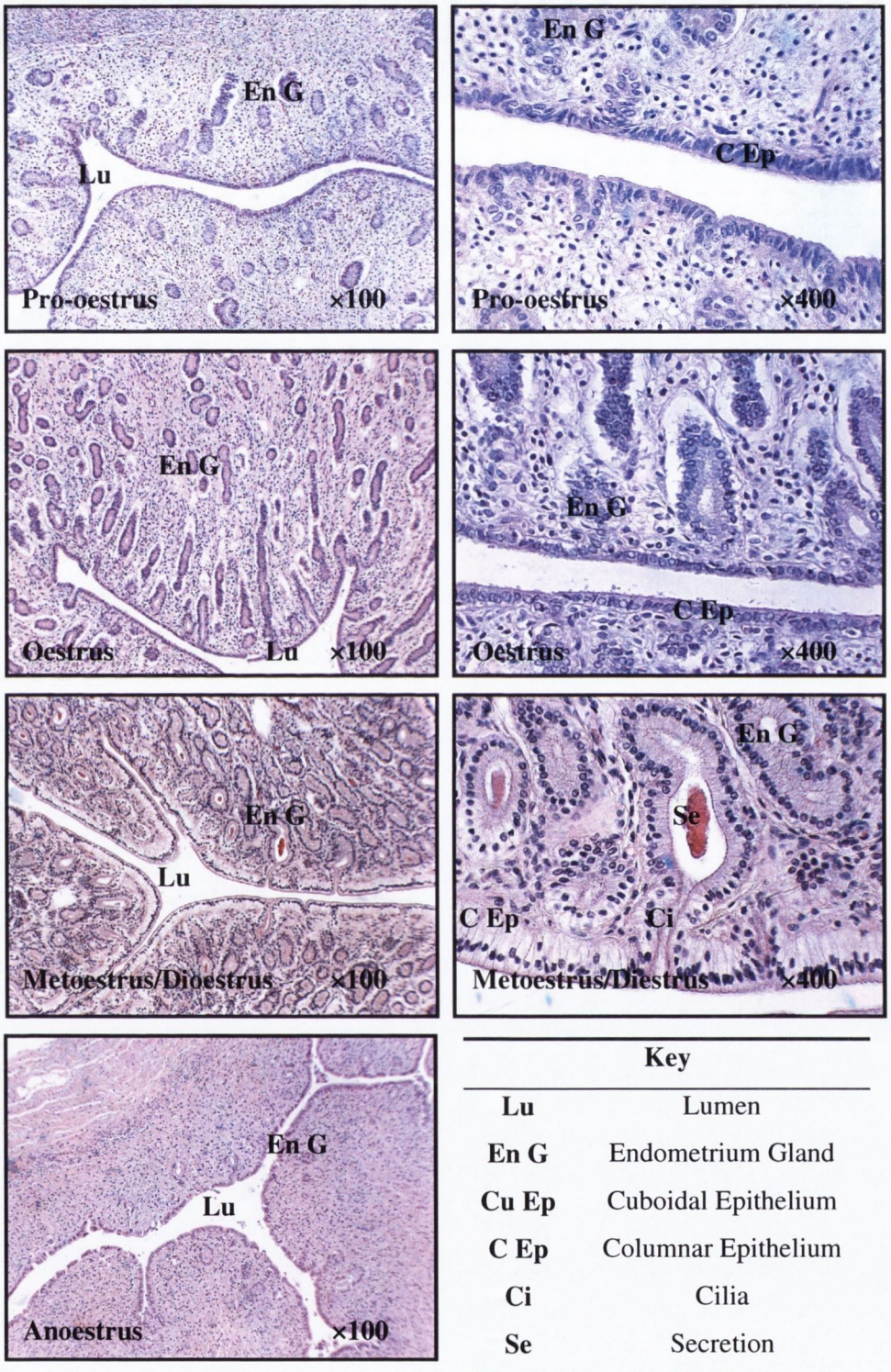
Uterine Endometrium	Description
Pro-oestrus	Endometrium quite thick, simple columnar luminal epithelium, developing glands enlarged and located at the interior edge of the endometrium
Oestrus	Endometrium thick, simple columnar luminal epithelium, highly developed glands, fully enlarged and located at the interior edge and periphery of the endometrium
Metoestrus/Dioestrus	Endometrium very thick, simple columnar luminal epithelium, fully developed glands, enlarged with occasional pink secretion, glands dispersed throughout endometrium
Anoestrus	Endometrium thin, simple cuboidal luminal epithelium, regressing/underdeveloped glands, small and sparsely dispersed throughout endometrium

The glands of the uterine endometrium show a progressive development through the oestrous cycle from pro-oestrus to dioestrus; the glands increase in size and become more numerous and dispersed throughout the endometrium during the stages of the cycle (Plate 2-1). The endometrium of anoestrual females show no glandular development. It was possible to clearly identify proestrus, oestrus, and anoestrus periods but differentiation between metoestrus and dioestrus was not possible so these two stages were combined (Table 2-1).

The vaginal epithelium becomes more stratified with keratinised cells as the oestrous cycle advances from proestrus to oestrus (Plate 2-2). These two stages were easily identified but it was difficult to differentiate between metoestrus, dioestrus and anoestrus so these three stages were combined .The thickness of the vaginal epithelium was measured using a graticule (1 unit = 0.25μm at ×400 magnification).As the measurement was very small in value it was multiplied by 10^2 and represented as $10^{-2}mm^2$.

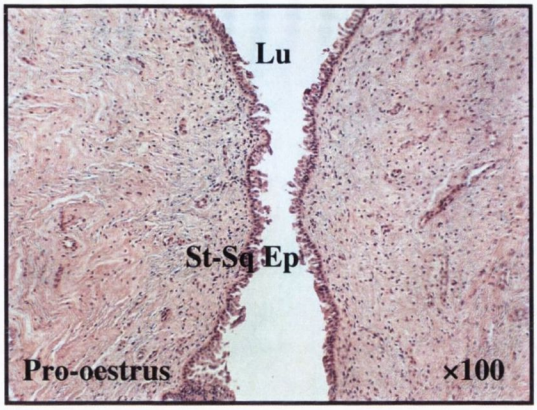
Table 2-2: Histological description of the vaginal epithelium used for identification of stages in the oestrus cycle; adapted from (Bacha & Bacha, 2000) and (Dellmann & Eurell, 1998).

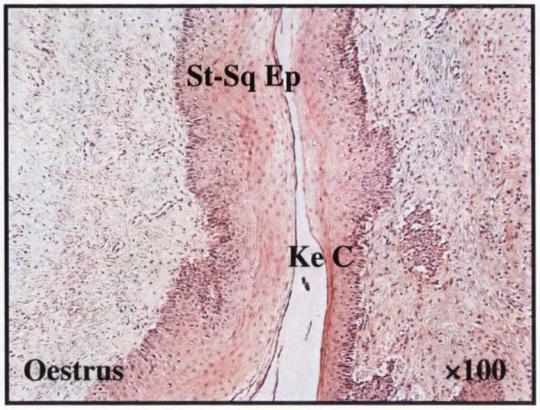
Vaginal Epithelium	Description
Pro-oestrus	Thin (<3 layers) stratified squamous epithelium, keratinsed cells may be present
Oestrus	Very thick (>3 layers), stratified squamous epithelium, keratinised cells at surface and can be visible in the vaginal lumen as exfoliated cells
Metoestrus/Dioestrus/Anoestrus	Very thin, epithelium ranging from stratified squamous to stratified cuboidal

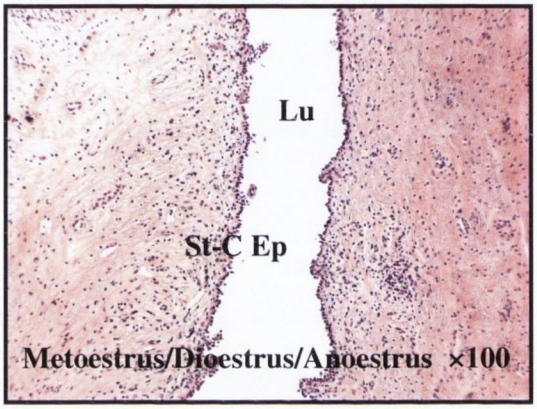


Key	
Lu	Lumen
En G	Endometrium Gland
Cu Ep	Cuboidal Epithelium
C Ep	Columnar Epithelium
Ci	Cilia
Se	Secretion

Plate 2-1: The uterine endometrium as it progresses through the stages of the oestrous cyc







Key	
St-Sq Ep	Stratified Squamous Epithelium
St-C Ep	Stratified Cuboidal Epithelium
Ke C	Keratinised Cells
Lu	Lumen

Plate 2-2: The vaginal epithelium as it progresses through the stages of the oestrus cycle.

2.2.4 Histological Examination of Ovaries

Prior to ovulation, a number of stages in folliculogenesis are histologically visible within the stroma of the ovary. Primordial follicles are always present as these are the precursor stage. In response to hormonal stimulation and a process of recruitment and selection, cohorts of primordial follicles undergo a series of changes in size and cellular composition during each oestrous cycle. Of this cohort of developing follicles only a certain number of pre-ovulatory follicles will undergo complete development, culminating in their rupture and release of mature oocytes, denoting ovulation. The remaining follicles undergo degeneration, atresia, at various stages in their development producing arteric follicles (Dellmann & Eurell, 1998, Senger, 1997, Sternberg, 1997).

The first stage in maturation, resulting in the formation of primary follicles, involves enlargement of the oocyte and development of the surrounding epithelium to simple cuboidal follicular cells (Dellmann & Eurell, 1998, Sternberg, 1997). The epithelium becomes a stratified granulosa layer of three to five layers which surround the primary oocyte, signifying the development of the secondary follicle. Further development of the secondary follicle produces a homogenous acellular layer which encompasses the oocyte known as the zona pellucida. During the development of the tertiary/graafian follicle, the surrounding ovarian stromal cells differentiate into several layers of theca interna cells and an outer layer of theca externa cells. Secretion by these cells eventually results in the production of a large hollow or antrum. Both the oocyte and the follicle itself continue to increase in size and the oocyte assumes an eccentric position within the follicle surrounded by layers of granulosa cells. The dominant follicles will continue to increase in size, when fully matured they will protrude from the ovarian surface and will eventually rupture owing to a series of physical changes to both the follicle wall and the surface of the ovary (Sternberg, 1997)

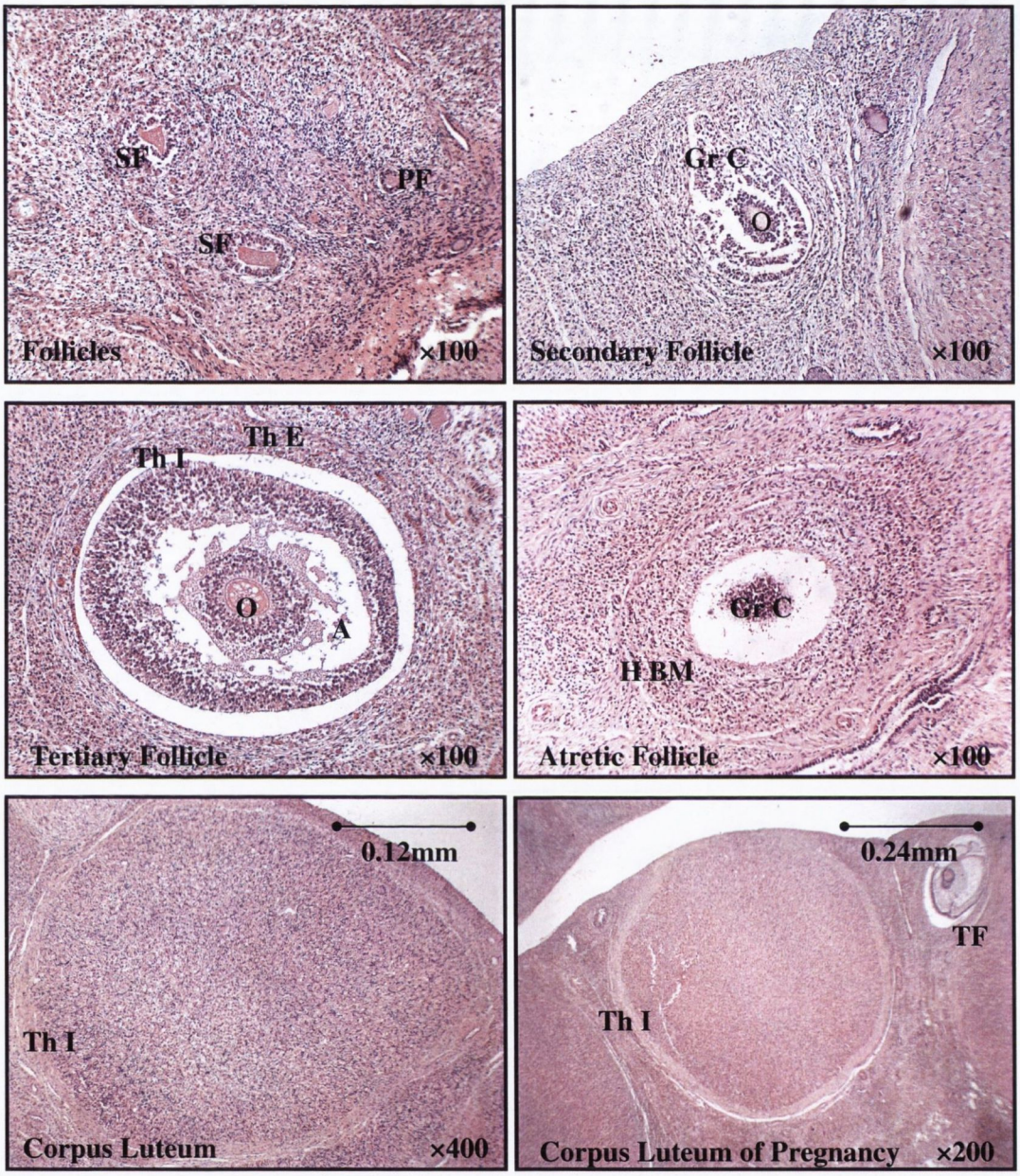
Following ovulation a corpus luteum forms from the remaining follicle cells; initially being called the corpus hemorrhagicum because of its bloody appearance (Senger, 1997). Granulosa cells and the cells of the theca interna multiply, increase in size, and differentiate into granulosa lutein cells and smaller theca lutein cells; respectively (Bacha

& Bacha, 2000). Delayed implantation is associated with reduced luteal activity owing to incomplete differentiation of the luteal cells (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981, Mead, 1981, 1993, Renfree & Calaby, 1981). The relatively inactive corpora lutea are poorly vascularised; the luteal cells are small, have irregular nuclei and appear to be lacking any secretory apparatus. Prior to implantation the corpora lutea increase in diameter, luteal cells increase in size and the secretory apparatus becomes more prominent (Canivenc & Bonnin, 1981)

The sections of the ovary were examined at ×40 and ×100 magnifications. Pre-ovulatory follicles were identified as secondary or tertiary follicles (Table 2-3) (Plate 2-3). In each section the follicles were counted and their diameters (the two greatest diameters at right angles to each other) were measured using a graticule. Arteric follicles were also identified, counted and measured.

Table 2-3: Histological description used for identification of reproductive follicles located on the ovaries; adapted from (Sternberg, 1997) and (Bacha & Bacha, 2000).

Follicle Type	Description
Secondary	2-3 stratified layers of granulosa cells, zona pellucida surrounding the oocyte, no antrum, oocyte centrally positioned
Tertiary	Several theca interna layers, ill-defined theca externa layer, large antrum, eccentric position of the oocyte
Corpus Luteum	Large, irregular theca interna constitute the epithelium, luteal cells of varying size are present throughout, no oocyte
Atretic	Degeneration and exfoliation of granulosa cells, wavy eosinophilic hylanised basement membrane



Key			
PF	Primary Follicle	**A**	Antrum
SF	Secondary Follicle	**Th I**	Theca Interna
TF	Tertiary Follicle	**Th E**	Theca Externa
O	Oocyte	**HBM**	Hylanised Basement Membrane
Gr C	Granulosa Cells		

Plate 2-3: Reproductive follicles located on the ovary

Each section of the ovary was examined for the presence of corpora lutea (Table 2-3) (Plate2-3). The corpora lutea were counted and the two largest diameters were measured using a graticule (1 unit = 2.5μm at ×40 magnification)

The corpora lutea were generally ovoid or elliptical in shape and not circular, therefore, the following equation was used to calculate the surface area:

$$\text{Surface Area} = \pi\left(\frac{d_1}{4} + \frac{d_2}{4}\right)^2$$

Where d_1 = diameter 1, and d_2 = diameter 2

As the surface area was very small in value it was multiplied by 10^2 and represented as $10^{-2}mm^2$.

2.2.5 Histological Examination of Mammary Glands

Cross sections of the mammary gland were examined for evidence of lactation at ×100 and ×200 magnifications. The features examined were the thickness of the secretory area (area containing lobules), the length/size of the teat, the relative thickness of the interlobular connective tissue, the expansion in diameter of the secretory units and the presence of pink/purple secretion in the secretory units of the lobule. Females were assigned to one of four derived categories (

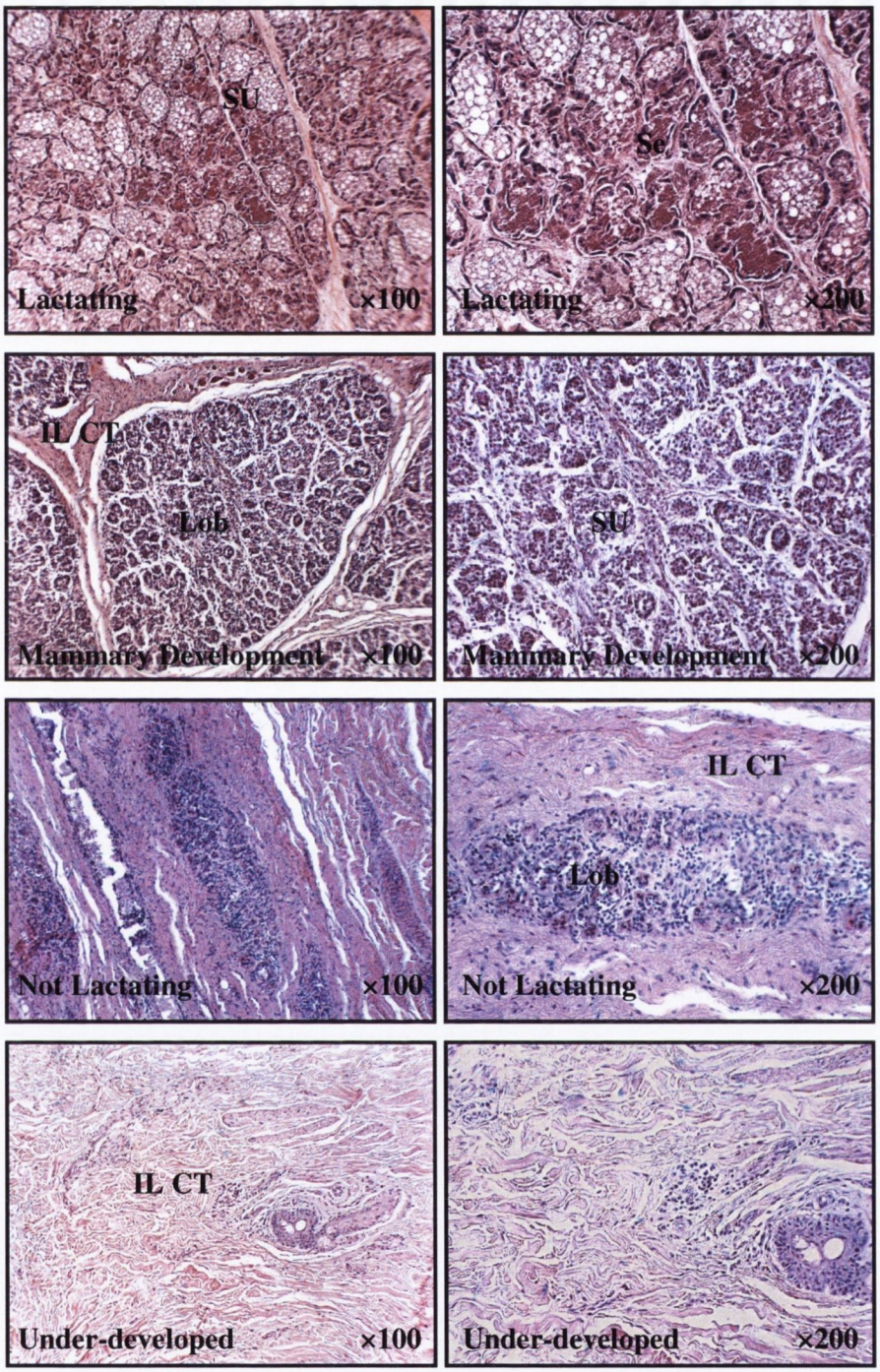
Table 2-4) (Plate 2-4). Lactating females had developed mammary tissue and enlarged secretory units suggesting active secretion (pregnant or recently parturient females). Females with mammary development did not have enlarged secretory units and had no active secretion (pre- or post- lactation). The mammary tissue of females not lactating had evidence of a previous period of lactation but was fully involuted (failed breeding season) and those that were under-developed had no mammary tissue present (prepubescent females).

Table 2-4: Histological description of the mammary glands used to assign females to categories of lactation; adapted from (Bacha & Bacha, 2000).

Category	Description
Lactating (1)	Thick secretory area, elongated teat, reduced interlobular connective tissue, secretory units expanded with the occasional presence of pink/purple secretion.
Mammary Development (2)	Thick secretory area, teat slightly elongated, reduced interlobular tissue, no expansion of secretory units and no evidence of pink/purple secretion.
Not lactating (3)	Secretory area reduced, teat involuted, thick interlobular connective tissue, no expansion of secretory units and no evidence of pink/purple secretion
Under-developed (4)	No secretory area or lobules visible

2.2.6 Circulating Progesterone Estimation

Blood samples were stored at 5℃ for 24 hours prior to serum extraction. The samples were centrifuged at a g-force of 1389 for 10 minutes. Between 1-3ml of serum was collected and although the blood was, in general, heavily haemolysed it was still suitable for analysis of progesterone levels. The serum samples were stored at −25℃. A radioimmunoassay for progesterone, following validation, was conducted at the Hormone Assay Laboratory – Veterinary & Animal Bio-Sciences Section in University College Dublin using AutoDELFIA progesterone kits (Manufacturer: PerkinElmer Life and Analytical Sciences, Turku, Finland)



Key			
Lob	Lobule	**SU**	Secretory Unit
IL CT	Interlobular Connective Tissue	**Se**	Secretion

Plate 2-4: Stages of development in the mammary gland

2.3 Examination of the Male Tract

2.3.1 Gross examination

The testes including the epididymis were weighed (nearest 0.01g) and the diameter was measured at the widest point (nearest 0.01mm) using an electronic calliper. From November to January the testes appeared to become smaller in size and encased in a thicker layer of fat, therefore, the excess fat was removed prior to weighing and measuring. The weight of the seminal vesicles was recorded, as was the weight and length of the baculum. Any abnormalities were noted. All the samples, excluding the baculum, were stored in 10% formalin. The tissue surrounding the testes tends to be highly impermeable to formalin so a transverse section was made half way through the testes to allow for the internal tissues to be fixed fully.

2.3.2 Histological Processing

Three sections were taken from each testis. Transverse sections, approximately 1mm. in thickness, were made through the top of the testis and the caput epididymis, the centre of the testis and the corpus epididymis and the bottom of the testis and the cauda epididymis. The tissues were processed as described in Appendix I

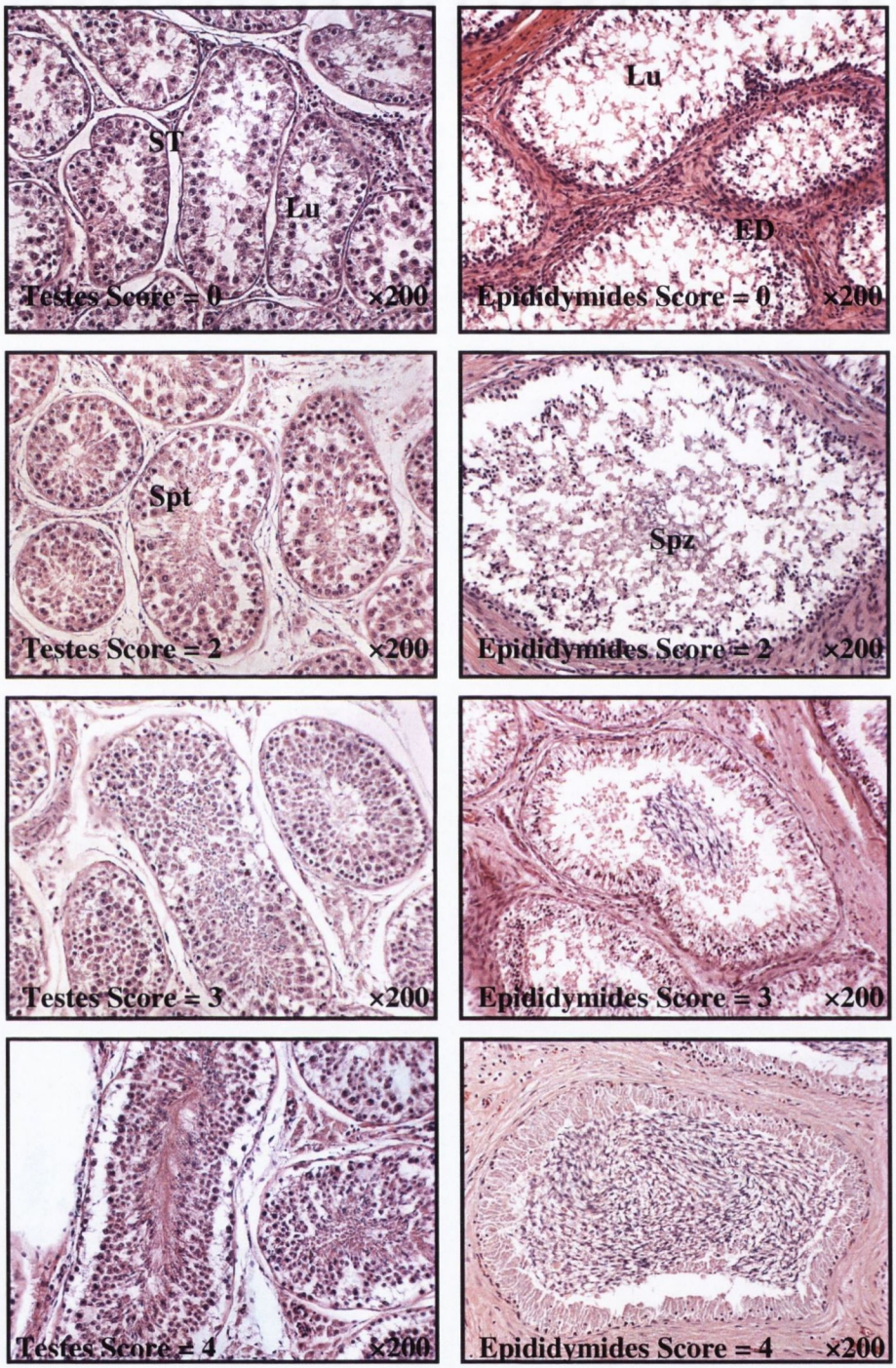
2.3.3 Histological examination of the Testes

Spermatogenesis, the development of spermatagonia into spermatozoa, occurs in the testes and the final stages of maturation and storage takes place in the epididymides. There are a number of stages in this step-wise process and all are visible in the seminiferous tubules of the testis. The cells at the different stages of development are referred to as the spermatagonia, spermatocytes, spermatids and finally the spermatozoa. Maturation of spermatozoa occurs in the caput and corpus epididymis and the mature spermatozoa are stored in the cauda epididymis (Senger, 1997).

The central sections of the testes and the cauda epididymides were examined at ×100, ×200 and ×400 magnifications for evidence of spermatogenesis and storage of spermatozoa, respectively. Spermatogenesis was determined to be active if all four cell types were present in the seminiferous tubules and inactive if the cell types of the final stages of development (spermatids and spermatozoa) were absent. The ducts of the cauda epidiymis were examined for the presence of mature spermatozoa and scored as present or absent. Once spermatogenesis was established, a further scale was created to score the abundance of spermatozoa in the lumen of the seminiferous tubules of the left testis and the ducts of the left cauda epididymis (Table 2-5) (Plate 2-5). It was possible to only score one of the testes as there was no variation in abundance between left and right. In those cases were the left testis was unavailable, that is, where it had been lost/damaged during the post mortem examination or it had been inappropriately processed during the histological procedure, the right was scored.

Table 2-5: Histological description used to assign abundance scores to males for spermatozoa presence in the testes and epididymis.

Score	Lumen of Semiferous Tubules (Testes)	Lumen of Epididymal Tubules (Epididymis)
0	Spermatozoa absent	Spermatozoa absent
1	Few sertoli cells associated with spermatozoa	Few spermatozoa present
2	Roughly half sertoli cells associated with spermatozoa	Clusters of spermatozoa
3	Majority of sertoli cells associated with spermatozoa	Dense clusters of spermatozoa
4	All sertoli cells associated with spermatozoa (layers visible)	Entire lumen filled with spermatozoa



Key			
ST	Seminiferous Tubule	**Spt**	Spermatid
ED	Epididymal Duct	**Spz**	Spermatozoa
Lu	Lumen		

Plate 2-5: Abundance scores for spermatozoa in the testes and the epididymides.

2.3.4 Analysis of Annual Hormonal Cycle – Circulating Testosterone

Blood samples were stored at 5℃ for 24 hours prior to serum extraction. The samples were centrifuged at a g-force of 1389 for 10 minutes. Between 1-3ml of serum was collected and although the blood was, in general, heavily haemolysed it was still suitable for the analysis of testosterone levels. The serum samples were stored at –25℃. A radioimmunoassay for testosterone, following validation, was conducted at the Hormone Assay Laboratory – Veterinary & Animal Bio-Sciences Section in University College Dublin using Amersham Testosterone kits (Manufacturer: GE Healthcare)

2.4 Demographics

2.4.1 Group Size

When a cattle herd contracts BTB it is referred to as a herd breakdown. As part of the control programme conducted by the Department of Agriculture, Fisheries and Food (DAFF), surveys are carried out to determine the possible source of infection of the breakdown. Where no alternative cause can be determined, it is assumed that a wildlife reservoir must be responsible. Badger setts are targeted within a 1km radius around the farm which experienced the breakdown. The intention of the culling program is to remove all badgers from these setts. Stopped restraints are used to capture badgers and are placed at setts for eleven consecutive nights. The restraints are generally placed at the entrance to setts and along well defined paths made by the badgers. All the badgers trapped at an individual sett are considered to be members of the one social group. Therefore, it was possible to assign individuals to specific groups.

Juveniles were not included in group size analysis as they were considered to be reproductively inactive and therefore not involved in reproductive competition.

2.4.2 Aging

Tooth wear was used to estimate the age of each badger, as it has been suggested to be one of the most accurate measures, based on results from a study of known-age material (Harris *et al.*, 1992). Individuals were classified into one of four age categories: Juvenile (<1 year), yearling (1-2 years), adult (2-4 years) and aged adults (> 4 years).

Table 2-6: Description of tooth wear parameters used to divide badgers into one of the four possible age categories; adapted from (Hancox, 1988).

Teeth	Juvenile	Yearling	Adult	Aged
Incisors	No dentine visible	Small areas of dentine visible on outer incisors	Large areas of dentine visible on all incisors	Large area of dentine visible, teeth flush with gum or missing
Canines	Very pointed	Slightly blunt, small area of dentine visible	Blunt, dentine visible, wear on inside of tooth	Very Blunt, dentine visible, flush or missing
Premolars	Not fully erupted, very sharp	Fully erupted, slightly blunt, small area of dentine visible	Blunt, dentine visible	Very blunt, large area of dentine visible, flush or missing
Molars	Cusps all present and sharp	Wear on some cusps	Wear on all cusps, whole sections worn	Heavy wear, tooth hollow or missing
Overall Colour	Very white	Starting to yellow	Yellow	Yellow, black (decay)

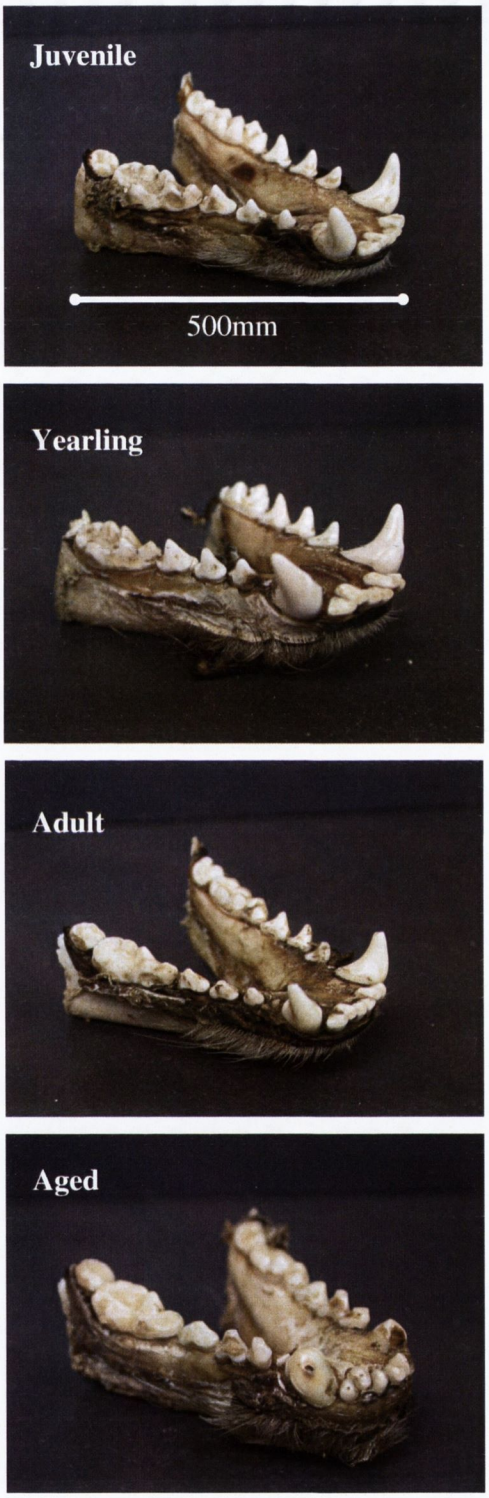


Plate 2-5: Mandibles showing progression of tooth wear through the 4 age categories

The mandible was collected from each badger and preserved in 10% formalin, ensuring that any *M. bovis* was killed. The mandibles were washed with water to remove the formalin and then left to dry in a fume cupboard. The degree of wear and discolouration of the incisors, canines, premolars and molars were assessed for both sides of the mandible and given a score of 1-4. This score allowed each badger to be placed into one of the four age categories (Table 2-6)

In addition, body length (mm), femur length (mm) and baculum length (mm), for males, were all recorded as described above. Comparisons were made between these measurements and the derived age categories to determine their possible function in age determination.

2.4.3 Body Condition

Three measurements of body condition were taken for each badger: body weight, the depth of the subcutaneous fat at the mid-lumbar region and the ratio of peri-renal (kidney) fat weight to kidney weight.

Adipose tissue provides a reserve of lipids or fats which can be converted to provide energy when required. It is located beneath the skin as subcutaneous fat, surrounds vital organs as visceral fat and is the yellow marrow component in bone marrow. As well as providing energy reserves, subcutaneous fat functions in insulation, fat associated with internal organs acts as protection and yellow bone marrow can be converted to red marrow during extreme blood loss or anaemia.

During times of reduced food abundance fats are converted to provide energy. Reserves are utilised from the subcutaneous region first, secondly from the fat that surrounds internal organs, and, finally, it is extracted from bone marrow. All badgers examined had some peri-renal fat so it was unlikely that fat was required from bone marrow reserves. Therefore, it was concluded that subcutaneous fat depth and peri-renal fat mass would be sufficient measures of body condition.

Body weight was recorded in kilograms, the depth of the subcutaneous fat was recoded in millimetres and the ratio of peri-renal fat weight to kidney weight was converted to a percentage kidney fat index, known as 'Riney's Kidney Fat Index'. The following equation was used to calculate the index (Riney, 1955):

$$Riney'sKidneyFatIndex = \left(\frac{perirenalfatweight}{kidneyweight(excl.fat)} \right) 100$$

2.4.4 Disease Status

As mentioned the project used a subset of the badgers captured as part of the national BTB control programme. Badgers were examined visually during the post mortem examination for BTB lesions by Dr. Ursuala Fogarty. The carcass, the head/neck, the abdominal cavity and the thoracic cavity were examined for lesions. In particular the following specific sites were examined: the skin, the prescapular, popliteal, pharyngeal, parotid, and submandible lymph node, the kidney, the spleen, hepatic and mesenteric lymph nodes, the liver, lung and mediastinal and bronchial lymph nodes. For health and safety reasons samples were not collected from badgers suspected to have generalised BTB and these individuals were excluded from the entire study.

The disease status of every badger involved in the study population was defined and they were divided into three categories: positive for BTB (visible lesions with the histological characteristics of BTB), inconclusive (visible lesions that lacked the characteristics of BTB) or negative for BTB (no visible lesions). The diagnosis of BTB was made by Dr. Ursuala Fogarty.

2.5 Statistical Analysis

The distributions of the data sets were investigated visually by production of histograms and Q-Q plots, and statistically by examination of skewness, kurtosis and the Kolmogrov-

Smirnov test for normality with Lilliefors significance correction. All plots and statistics were generated using *SPSS 15.0 for windows.*

To be of normal distribution, histograms had to have a bell shaped curve with little evidence of skewness. The data points of a normal Q-Q plot had to show little deviation from the plotted straight line and the points of the detrended Q-Q plot had to collect around the zero line and not be overly clustered.

Skewness and kurtosis values were within the boundaries of normality if they lay in the range of –1 to 1 and –0.7 to 0.7; respectively. The Kolmogrov-Smirnov test for normality with Lilliefors significance correction compared the data set to be tested with an expected normal data set. The significance level was set at $p<0.05$. Therefore, obtaining a p-value lower than this significance level implied that the data set was not normally distributed.

All of these tests for normality were taken into consideration. Data sets which complied with a majority of the tests were deemed to have normal distribution, and therefore, it was possible to use parametric methods for statistical analysis of the data. In addition, prior to statistical analysis the Levene's test for equality of variance was used to test the homogeneity of the variance between the test groups; the significance level was set at $p<0.05$. If the p-value obtained from the test was lower than the significance level the variance between groups was heterogeneous and non-parametric methods of statistical analysis were used.

Data sets which deviated from the tests for normality were considered to be not normally distributed and non-parametric methods of statistical analysis were used.

Outliers and extreme outliers were identified by creating boxplots in SPSS. Outliers are values between 1.5 inter-quartile ranges and 3 inter-quartile ranges from the end of the box (the length of the box corresponds to the inter-quartile range). Extreme outliers are values that are more than 3 inter-quartile ranges from the end of the box.

The breeding year is considered to begin in March with parturition and renewed oestrous cycles. Therefore, any analysis involving month or season followed this same pattern; starting with March and ending with February. Juveniles were not included in any analysis of the reproductive cycle as they were considered to be sexually immature.

3 DEMOGRAPHY AND MORPHOLOGY

3.1 Introduction

This chapter will focus on the demography and morphology of the study population. The demographics described include the geographic origin of each badgers (location), sex, age, and group size.

Density dependent constraints on fecundity and cub survival are an indirect response to limited resources in populations which are approaching carrying capacity. Limited resources may directly affect reproductive success by reducing body condition; alternatively a reduction in resource availability may increase breeding competition and reproductive suppression which in turn will reduce reproductive success. Within some populations only a single, possibly dominant, female may breed successfully in a social group (Kruuk, 1978, Revilla *et al.*, 1999) whereas multiple females are reported to breed in other populations (Carpenter *et al.*, 2005, Domingo-Roura *et al.*, 2003, Woodroffe & MacDonald, 1995b). Comparisons will be made between group sizes in Irish badgers and those of the UK and Europe to determine whether group size could have an effect on reproductive potential. The effect of group size on fecundity will be described in Chapter 5.

A definitive aging technique has not been described for badgers. Various practices have been reviewed (Hancox, 1988) and for this study tooth wear was used as a method of aging. A series of morphological measurements were collected during the study for both sexes: body length, hind foot length, and femur length; and baculum length and weight

for males. Some of these measurements were compared to ages derived from tooth wear scores (see Section 2.4.2) to determine whether they could be useful in age classification.

Although gestation is not associated with a reduction in body condition, lactation can cause substantial losses (Woodroffe & MacDonald, 1995a). The majority of females recover from these reductions in body condition by autumn, but there is a high age-specific mortality associated with lactation, with two-year-old breeders being less likely to survive than non-breeders of the same age (Woodroffe & MacDonald, 1995a). Body condition has a significant effect on reproductive success and timing. Variations in body condition follow an annual pattern, with fat deposition occurring at its highest levels during autumn (Kruuk & Parish, 1983). High fat reserves are necessary to counteract the reduction in body condition which occurs during lactation. Females in relatively poor condition in autumn, prior to breeding, were more likely to fail in subsequent gestations; producing no cubs in the spring. This may be a control against potential mortality owing to the costly period of lactation and/or a response to low food availability (Delahay *et al.*, 2006a, Woodroffe & MacDonald, 1995a, Woodroffe & MacDonald, 1995b). In addition, body condition has been reported to be influential in the timing of implantation, with females in relatively high body condition implanting earlier in the season (Woodroffe, 1995). The annual cycle of varying body condition will be described for the male and female badger. These variations will be related to reproductive success in Chapter 5.

Although tuberculosis is endemic within the badger population both in the UK (Clifton-Hadley *et al.*, 1993) and Ireland (O'Boyle *et al.*, 2003) it may not increase mortality in all cases (Swinton *et al.*, 1997, Wilkinson *et al.*, 2000). Immunity and progression of *Mycobacterium bovis* infection appears to vary between individuals, in particular males may be more likely to show rapid disease progression compared to females (Rogers *et al.*, 2003, Wilkinson *et al.*, 2000). Chronically infected individuals can survive for extended periods of time and are capable of successful reproduction and lactation (Clifton-Hadley *et al.*, 1993). However, individuals in the progressive stage of the disease experience poor body condition and extreme weight loss resulting in higher levels of mortality than uninfected individuals (Clifton-Hadley *et al.*, 1993, Wilkinson *et al.*, 2000). The intention

of this area of the study was to determine if disease status was related to demographic features.

3.2 Results

3.2.1 Study Population

3.2.1.1 Description

Badger tissues were collected between March 2005 and March 2006; and between June and September 2006. The total sample size for the 17 months was 620 individuals. As the procedure for tissue collection and processing was not finalised until after the first month of the study, that is March 2005, it was not possible to use all the tissues collected for this month. Therefore, a random sub-sample of correctly processed individuals was taken from the sample of March 2005, and combined with March 2006 to provide a sample size which was comparable to other months of the year. A total of 580 individuals were used in the demographic analysis of the study population.

Of these 580 individuals, 436 were allocated appropriate sett numbers (juveniles were not included in group size analysis). The study population were assigned to 293 setts or groups.

3.2.1.2 Demography of the Study Population

The demography of the monthly sample is described in Table 3-1. The geographic derivation of the study population was uniform, with 44% belonging to the northern region and 56% to the southern region. It was not possible to use the geographical division in further analysis due to sample size limitations when other factors were introduced.

Table 3-1: Demographics of the monthly sample collected between March 2005 and September 2006.

Location	Age	Jan		Feb		Mar		Apr		May		June		July		Aug		Sept		Oct		Nov		Dec		Yearly Total	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
North	Juvenile	3	2	0	0	0	0	0	0	0	0	1	1	2	1	1	0	0	0	1	0	3	0	1	0	12	4
	Yearling	8	7	8	11	4	4	8	6	4	3	1	4	2	1	3	0	2	0	2	6	5	1	3	1	50	44
	Adult	12	10	13	2	10	4	3	6	7	3	3	3	0	1	0	2	3	3	3	4	0	1	6	1	60	40
	Aged	3	2	5	1	5	5	3	4	0	1	1	3	0	1	0	1	0	0	0	1	2	4	0	3	19	26
	Total	26	21	26	14	19	13	14	16	11	7	6	11	4	4	4	3	5	3	6	11	10	6	10	5	141	114
South	Juvenile	1	1	0	1	0	0	0	0	0	0	0	0	2	0	3	0	0	0	1	0	1	0	3	0	11	2
	Yearling	7	5	10	9	9	3	7	2	2	4	2	2	5	4	6	5	4	8	3	5	5	0	5	5	65	52
	Adult	2	3	19	15	8	11	3	3	2	3	4	1	3	5	5	2	2	2	1	5	3	1	7	7	59	58
	Aged	2	3	8	7	7	9	0	1	4	0	2	1	2	3	2	3	5	1	3	4	1	1	3	6	39	39
	Total	12	12	37	32	24	23	10	6	8	7	8	4	12	12	16	10	11	11	8	14	10	2	18	18	174	151
Monthly Total		71		109		79		46		33		29		32		33		30		39		28		51		580	

The male to female sex ratio for the study population was 1.2:1, this deviated significantly from that of unity or a sex ratio of 1:1 (Chi-squared test: $\chi^2=4.31$; d.f.=1; $p<0.05$). Yearlings and adults made up the greatest proportions of the sample at 36.38% and 37.41%, respectively, aged individuals made up 21.21% and juveniles only 5% (Table 3-1). Juveniles were only caught between June and February. This may be expected as juveniles are not fully weaned until approximately 15 weeks, spending much of their time below ground (Neal & Cheeseman, 1996). By 8/9 months they have reached their full adult weight (Page *et al.*, 1994), making them large enough to be readily caught in the restraints. Juveniles were excluded from all analysis except the examination of age parameters and disease status, where they were analysed separately.

3.2.1.3 Group Size

Minimum group sizes ranged from solitary animals to nine individuals (mean=1.6), with all groups >4 individuals being mixed sex (Figure 3-1). The majority of badgers were solitary, with paired individuals being the most numerous type of group to be formed.

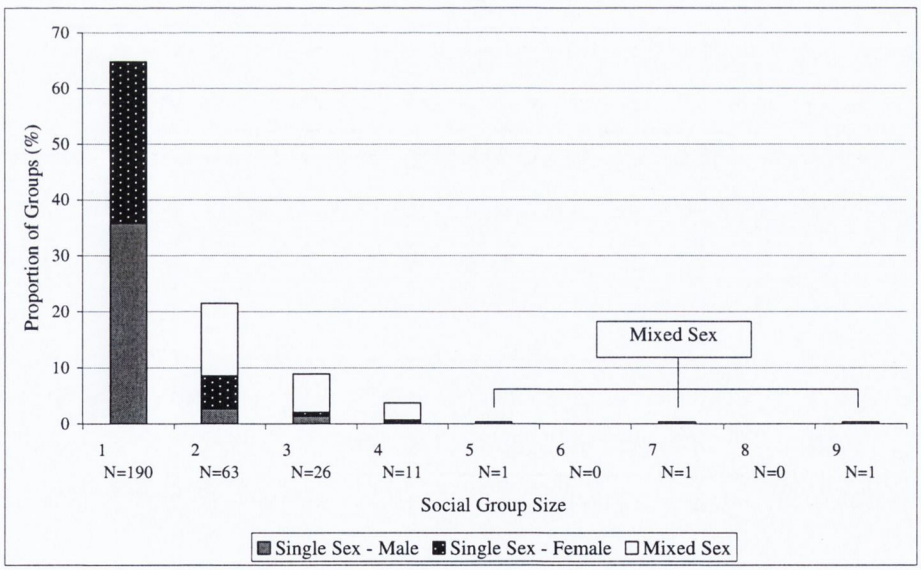


Figure 3-1: Proportion of different group sizes, as a percentage of total number of groups within the study population.

Solitary animals made up the highest proportion of groups, 64.85%. Of these individuals 55.27% were male; there was no bias for one sex to be solitary (Chi-squared test: χ^2=2.11; d.f.=1; p>0.05). It is also worth noting that bite wounding was only observed in three individuals during the entire study (0.5%; N=580). Single sex groups (>1 individual) were more common for females than males; as group size increased, the proportion of single sex groups reduced. There were few examples of groups of greater than four individuals, and all such groups were mixed sex.

3.2.2 Age Parameters

3.2.2.1 Description

Each individual was assigned an age category based on tooth wear (see Section 2.4.2). Body length and femur length for all badgers, and baculum length for males, were all compared to these derived age categories to identify possible correlations and, therefore, their potential function in age determination. In addition, comparisons were made between hind foot length and body length, and baculum weight and baculum length.

Body length and femur length both had normal distributions and had samples of N=549 (juveniles, N=28) and N=543 (juveniles, N=29), respectively. Parametric statistics were used in analysis of these data sets. The three data sets, hind foot length, baculum length and baculum weight, were all not normally distributed and had samples of N=547 (juveniles, N=26), N=289 (juveniles, N=23) and N=289 (juveniles, N=23), respectively. Therefore, non-parametric statistics were used to analyse these data sets.

3.2.2.2 Body length and Hind foot length

The range of body lengths, excluding extreme outliers (cut-off male: <730 and >940mm, n=3; cut-off female: <710 and >920mm, n=1; for criteria see Section 2.5), was 730-890mm for males and 710-885mm for females (juveniles: males = 625-880mm; females = 645-820mm). The range of hind foot lengths, excluding extreme outliers (cut-off male: <94 and >108mm, n=22), were also comparable between the sexes; 94-108mm for males and 90-105mm for females (juveniles: males = 90-105mm; females = 90-100mm).

However, mean body length (males = 831.49 ± 1.71mm; females = 814.77 ± 1.72mm) and median hind foot length (males = 100mm; females = 98mm) were significantly higher for males compared to females (t-test, body length: t=-6.876; d.f.=547; p<0.001; Mann Whitney, hind foot length Z=-7.406; N=258, 259; p<0.001).

There was a trend for increasing hind foot length with increasing body length for both males and females (Figure 3-2). The two sexes had a significant positive correlation between hind foot length and body length (Spearman rank-order correlation: males, r_s=0.306; N=288; p<0.001; females, r_s=0.241; N=258; p<0.001). However, the R^2 values obtained were not high (males: R^2 =0.1079; females: R^2 =0.0531), suggesting that for males 10% of the variation in hind foot length was related to changes in body length and for females the variation was only 5%.

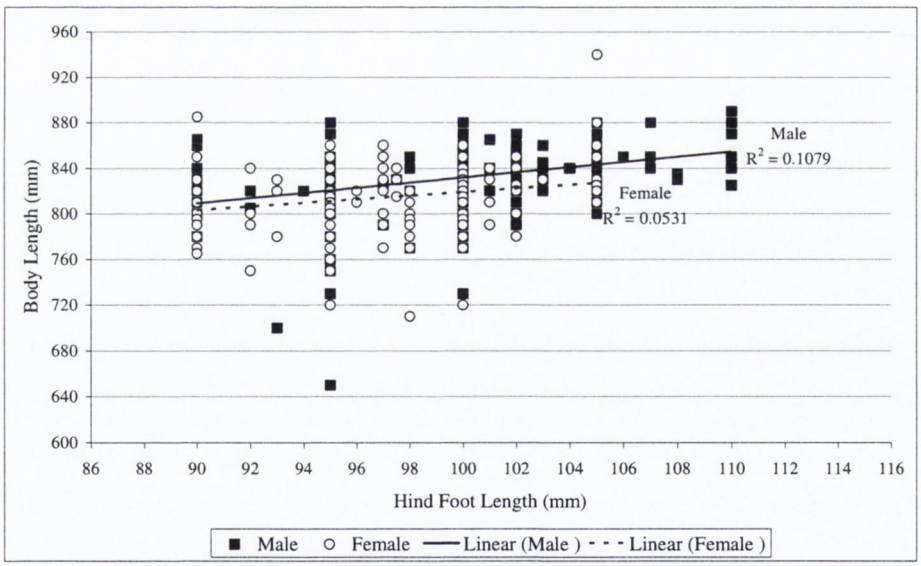


Figure 3-2: Distribution of hind foot length in relation to body length for male and female badgers.

There was a slight upward trend in body length in relation to age for both sexes (Figure 3-3). Variations in body length were greater for juveniles and yearlings and the maximum values were similar in all four age categories. There was significant positive correlation between the two parameters for females (Spearman rank-order correlation: r_s=0.125;

N=264; p=0.042), but the correlation was non-significant for males (Spearman rank-order correlation: r_s=0.061; N=313; p=0.279). However, for the former correlation the R^2 value was low (R^2=0.031) suggesting that there may not be a meaningful association between female body length and age.

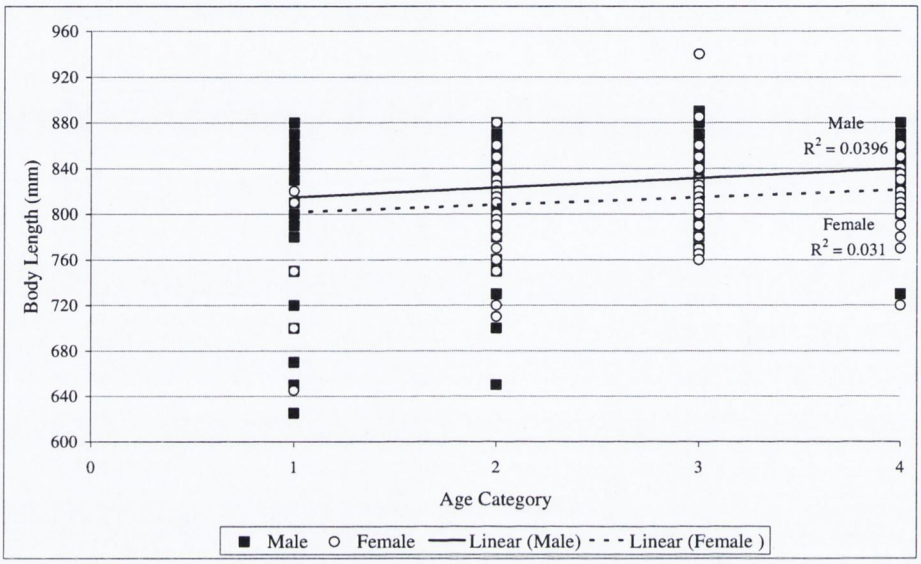


Figure 3-3: Distribution of body length in relation to the four age categories for male and female badgers. Where, 1=juveniles, 2=yearlings, 3=adults and 4=aged.

3.2.2.3 Femur Length

The range of femur lengths, excluding extreme outliers (cut-off males: <95.68 and >131.24mm, n=2; cut-off females <95.03 and >123.31mm, n=1; for criteria see Section 2.5), was 99.36-128.5mm for males and 98.74-121.31mm for females; (juveniles: male = 89.05-118.31mm; female =.81.49-110.93mm). Mean femur length was higher for males compared to females (males = 113.12 ± 0.25mm; females = 109.16 ± 0.23mm) (t-test: t=-11.721; d.f.=539.114; p<0.001).

There was little animal-to-animal variation in femur length for the four age categories (Figure 3-4). Femur length increased with progression through the age categories. There was a significant positive correlation between femur length and age for males but not for

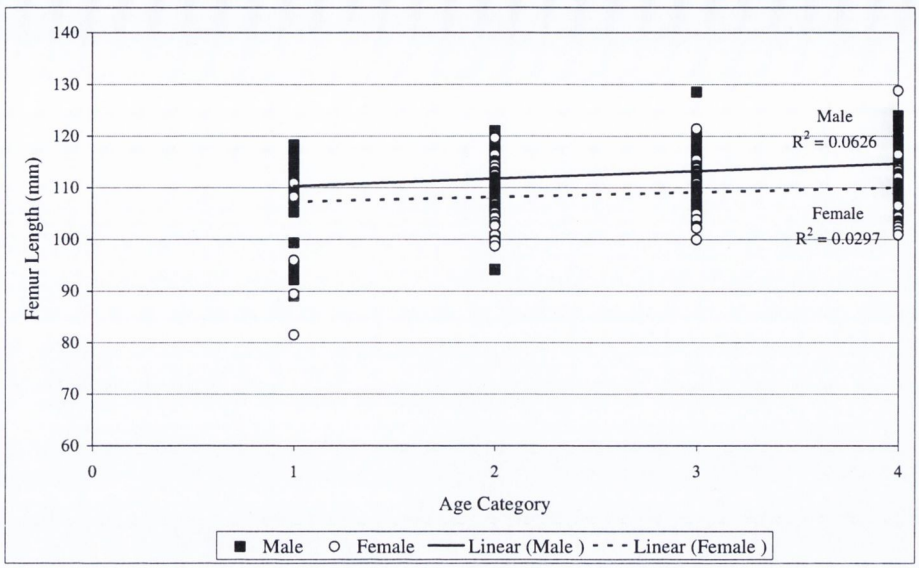


Figure 3-4: Distribution of femur length in relation to the four age categories for male and female badgers. Where, 1=juveniles, 2=yearlings, 3=adults, 4=aged.

females (Spearman rank-order correlation: males, r_s=0.151; N=308; p=0.008; females, r_s=0.092; N=262; p=0.139). However, the former correlation had a low R^2 value (R^2=0.0626), suggesting that only 6% of the variation in femur length was related to the age categories.

3.2.2.4 Baculum Dimensions

Baculum length increased with increasing baculum weight (Figure 3-5) providing a significant positive correlation (Spearman rank-order correlation: r_s=0.577; N=288; p<0.001). The R^2 value was high (R^2=0.3465), suggesting that over a third of the increase in baculum length was associated with an increase in baculum weight.

There was a trend for increasing baculum length with progression through the age categories (Figure 3-6). Animal-to-animal variation was lowest for aged males. There was a significant positive correlation between length and age (Spearman rank-order correlation: r_s=0.368; N=312; p<0.001), and the R^2 value (R^2=0.1662), suggested that nearly a 6th of the variation in baculum length was related to the age categories.

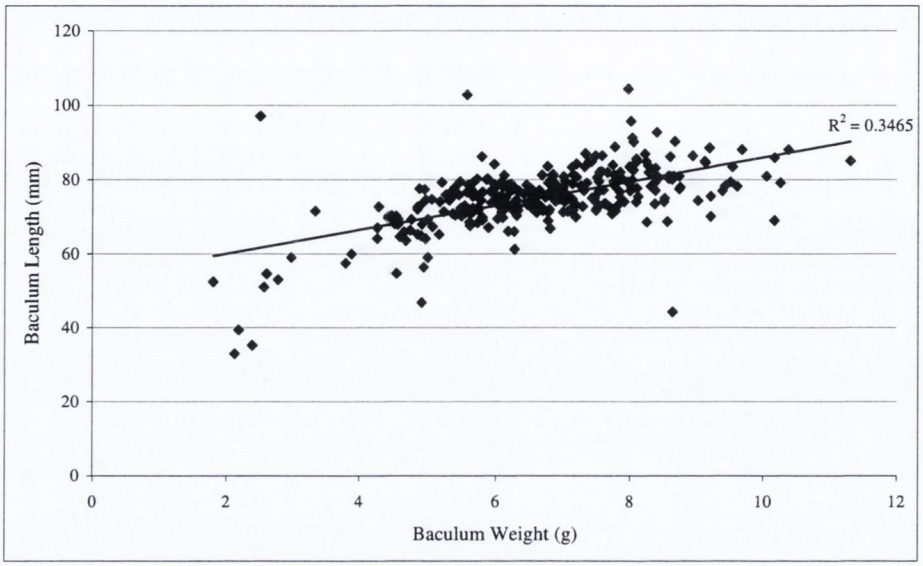


Figure 3-5: Distribution of baculum length in relation to baculum weight for male badgers.

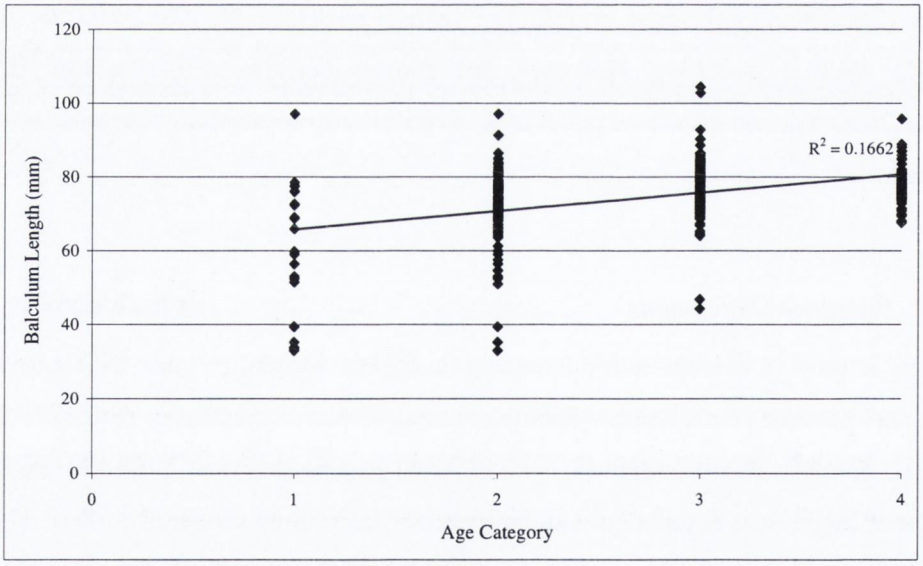


Figure 3-6: Distribution of baculum length in relation to the four age categories for male badgers. Where, 1=juveniles, 2=yearlings, 3=adult and 4=aged.

3.2.3 Body Condition

3.2.3.1 Description

Three measures of body condition were collected during the study: body weight, subcutaneous fat depth and the ratio of peri-renal fat weight to kidney weight. Body weight was recorded for 545 individuals; the distribution of the data set was normal allowing for parametric statistical analysis. In examples where compared sub-groups did not share equal variance, non-parametric statistical tests were used.

The ratio of peri-renal fat weight to kidney weight was converted to a percentage kidney fat index and was calculated for 546 individuals. Subcutaneous fat depth was recorded for 545 individuals. The distributions of these two data sets were not normal, therefore, non-parametric methods of analysis were used.

3.2.3.2 Body Weight

Monthly variations in body weight were parallel for male and female badgers (Figure 3-7). Male body weights were consistently higher than those of females and there was a significant difference in mean weight between the sexes (male = 9.74 ± 0.94kg; female = 9.05 ± 0.10kg), with males being higher (t-test: t=-5.024; d.f.=543; $p<0.001$).

For the first half of the reproductive year, March-August, body weight remained relatively constant for the two sexes (Figure 3-7). There was subsequently an increase in body weight with the greatest weights occurring in November-December. Body weights declined through January-February such that weights in February were similar to those recorded in March. The highest mean monthly body weights (males = 12.03 ± 0.24kg in December and females = 11.63 ± 0.55kg in November) were approximately 50% greater than the lowest mean weights recorded during the year (males = 8.34 ± 0.39kg in July and females = 7.63 ± 0.18kg in May).

There was a significant variation in body weight by month for both male and female badgers (Figure 3-7) (Kruskall Wallis: males, $\chi^2=142.140$; d.f.=11; $p<0.001$; females,

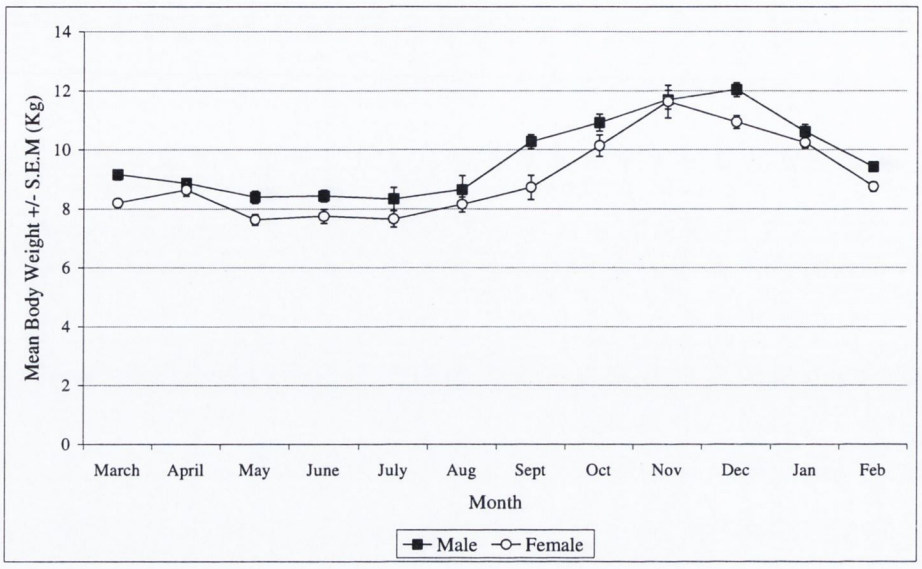


Figure 3-7: Variations in mean body weight by month for male and female badgers. Error bars represent standard error of mean.

χ^2=127.411; d.f.=11; p<0.001). The majority of months had low animal-to-animal variation; the months with the highest animal-to-animal variation were July-August for males and September-November for females and the lowest were February-June for males and December-May for females. The highest body weights for males were in November-December and were significantly higher than the months February-August (Dunn's post hoc test: p<0.001 in all cases). The changes in female body weight mirrored that of the males with a peak in November that was significantly greater than the months, February-August (Dunn's post hoc test: Nov vs. Feb & Apr, p<0.05; Nov vs. Aug, p<0.01; p<0.001 in all other cases). Mean female body weight in December was not as high as November, but with low animal-to-animal variation it was significantly higher than an additional month, February-September (Dunn's post hoc test: Dec vs. Sept, p<0.01; p<0.001 in all other cases).

3.2.3.3 Subcutaneous Fat Depth and Kidney Fat Index

Monthly variations in subcutaneous fat depth (Figure 3-8) and kidney fat index (Figure 3-9) followed parallel trends for the two sexes. Subcutaneous fat depths (S.F.D) and

kidney fat indexes (K.F.I) were consistently greater for females compared to males. Median fat depths and indices were significantly higher in females (S.F.D: male = 5mm; female =7.25mm, K.F.I: male = 33.35%; female = 47.77%) (Mann-Whitney: S.F.D, Z=-4.036; N=258, 287; p<0.001; K.F.I, Z=-5.026, N=256, 290; p<0.001).

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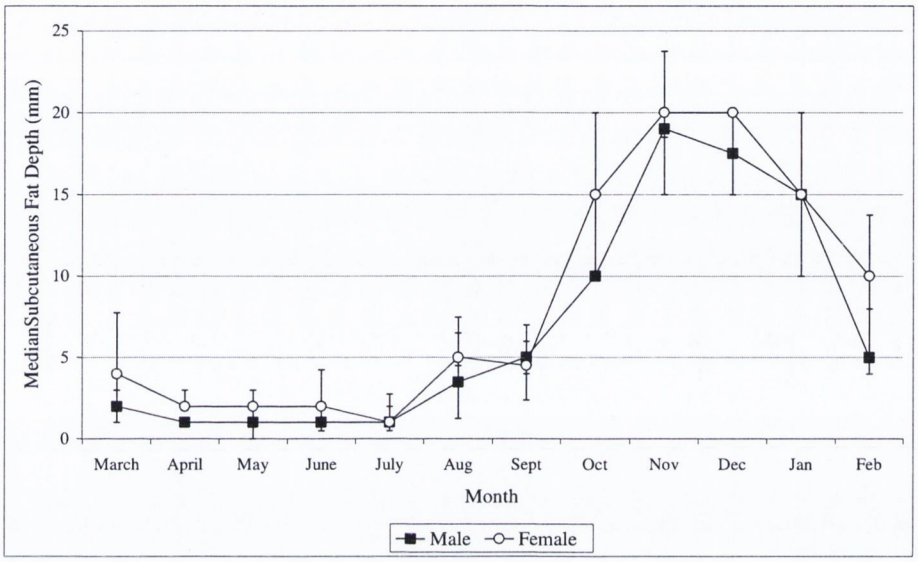

Figure 3-8: Variations in median subcutaneous fat depth by month for male and female badgers. Error bars represent inter-quartile range.

For the first six months of the reproductive year, March-August, subcutaneous fat depth (Figure 3-8) and kidney fat index (Figure 3-9) remained quite constant for the two sexes. There was subsequently a peak in these two parameters during the following six months, with the greatest values occurring in November-January. Median subcutaneous fat depth, at its highest values, was twenty times greater than the lowest median values (males: highest = 19mm in November, lowest = 1mm in April-July; females: highest = 20mm in November-December, lowest 1mm in July); kidney fat index was six times greater (males: highest = 86.28% in December, lowest = 16.05% in July; female: highest = 98% in January, lowest = 16.25% in July).

There was a significant variation in both subcutaneous fat depths and kidney fat indices by month for both male and female badgers (Kruskall Wallis, S.F.D.: males, χ^2=213.466;

d.f.=11; p<0.001; females, χ^2=194.104; d.f.=11; p<0.001; Kruskall Wallis, K.F.I.: males, χ^2=187.621; d.f.=11; p<0.001; females, χ^2=150.930; d.f.=11; p<0.001)

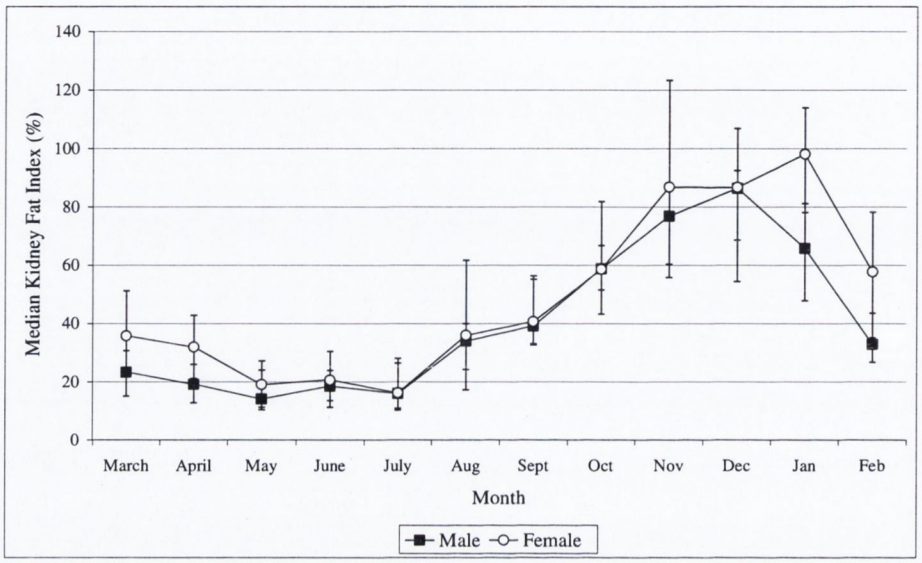

Figure 3-9: Variations in median kidney fat index by month for male and female badgers. Error bars represent inter-quartile range.

For females the peak in subcutaneous fat depth was in November-December (Figure 3-8) being significantly higher than the months March-September (Dunn's post hoc test: Nov vs. Aug, p<0.01; p<0.001 in all other cases). January had the highest kidney fat indices (Figure 3-9) for females being significantly greater than the months February-September, excluding July (Dunn's post hoc test: Jan vs. Feb, p<0.05; Jan vs. Sept, p<0.01; p<0.001 in all other cases).

The peak in subcutaneous fat depth (Figure 3-8) and kidney fat index (Figure 3-9) for males was between November-December. The highest values in subcutaneous fat depth occurred in November (Figure 3-8), being significantly higher than the months February-September (Dunn's post hoc test: Nov vs. Sept, p<0.05; Nov vs. Feb, p<0.01; p<0.001 in all other cases). December had the highest kidney fat index (Figure 3-9), which was significantly greater than the seven months, February-August (Dunn's post hoc test: p<0.001 in all other cases).

3.2.4 Disease Status

3.2.4.1 Description

For health and safety reasons badgers suspected of having generalised BTB were entirely excluded from the study. Dr Ursuala Fogarty (IEC) determined the disease status of 535 inividuals from the study population to be positive, inconclusive or negative for BTB infection.

3.2.4.2 Proportion of BTB infection

Of the individuals examined for BTB infection (N=535) 8.22% had a definitive diagnosis for BTB, 43.18% had an inconclusive diagnosis and 48.60% were negative (Figure 3-10). Geographical division of the study population showed that the northern region had a higher proportion of positive BTB cases compared to the southern region, 10.68% and 6.31%, respectively. Male and female badgers had comparable proportions of positive, inconclusive, and negative BTB cases, with 8.71% of males and 7.66% of females being positive for BTB.

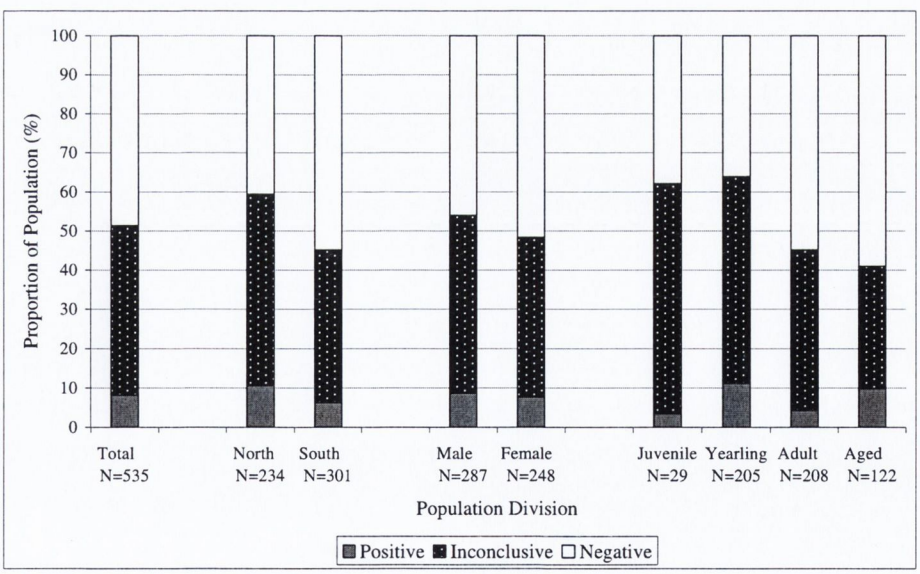

Figure 3-10: Breakdown of BTB diagnosis for the total population, north and south populations, male and female populations and the four age categories.

Yearling and aged adults had the greatest proportions of BTB cases, 11.22% and 9.84%, respectively (Figure 3-10); with juveniles having the least, 3.45%. Age category appeared to have an affect on BTB diagnosis. The proportion of inconclusive cases was highest for juveniles and reduced with progression through the age categories (juveniles, 58.62; yearlings, 52.68; adult, 40.87; aged, 31.15).

3.2.4.3 Location of BTB infection

Of the individuals found to be positive for BTB, N=42, visible lesions were found in six locations of the body: the prescapular lymph node, the popliteal lymph node, the pharyngeal/submandibular/parotid lymph nodes, the kidney, lungs, and the mediastinal/bronchial lymph nodes. Visible lesions were most prevalent in the pharyngeal/submandibular/parotid lymph nodes, then, in descending order of frequency, the popliteal lymph node, the mediastinal/bronchial lymph node and the prescapular lymph node, lungs and kidneys (40.48%, 38.09%, 35.71%, 33.33%, 16.67%, 4.76%; respectively) (Figure 3-11).

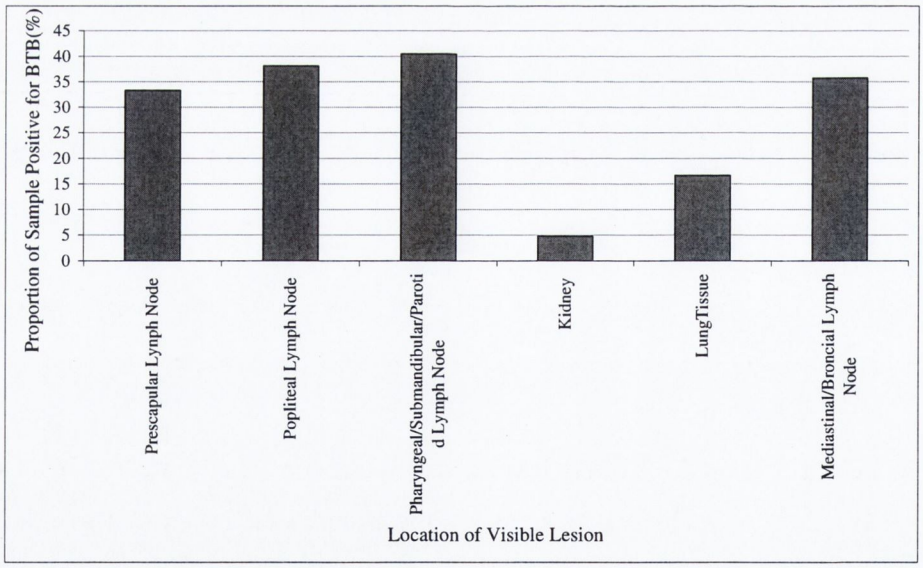

Figure 3-11: Proportion of individuals, as a percentage of the sample which were positive for BTB, to have visible lesions in one of the six locations of the body. Individuals could have lesions in >1 location.

3.3 Summary

- The male to female sex ratio for the study population (1.2:1) deviated significantly from unity.

- Yearling and adult badgers made up the greatest proportions of the study population.

- Minimum group size ranged from solitary animals to nine individuals with a mean group size of 1.6 individuals.

- Throughout the study extremely low levels of bite wounding were reported (0.5%).

- Body length, hind foot length and femur length were all significantly greater in males.

- There was little association between body length and hind foot length for both males and females. In males, baculum length had a reasonable association with baculum weight.

- Neither body length nor femur length were appropriate for use in age classification. Baculum length may be useful in separating juvenile males from the other three age categories: yearlings, adult and aged but only in conjunction with other aging methods

- Males had significantly higher body weights than females. Conversely, females had significantly higher subcutaneous fat depths and kidney fat indices.

- Body weight remained relatively low for males and females during the first 6 months of the breeding year (March-August). This was followed by an increase during the subsequent months to a peak in December for males and November for females. The peaks in body weight were approximately 50% greatest than the lowest weights.

- Female body condition scores, subcutaneous fat depth and kidney fat index, remained relatively low during the first 5 months of the breeding year (March-July). This was followed by quite a sharp increase in subcutaneous fat depth to a peak in November-December and a more gradual increase to a peak in January for kidney fat index scores. Male badgers followed a similar trend of changing body condition scores but the peaks occurred earlier in November for subcutaneous fat depth and December for kidney fat index scores. Subcutaneous fat depths at there highest values were twenty times greater then the lowest values and the peaks in kidney fat indices were six times greater than the lowest values.

- 8.22% of the study population tested positive for BTB and there was no geographical, sex or age biases for the infection.

- Visible BTB lesions were most commonly found in the pharyngeal/submandibular/parotid lymph nodes, the popliteal lymph node, the mediastinal/bronchial lymph nodes and the prescapular lymph nodes and least in the kidney and lungs.

3.4 Discussion

Although the sample of the population examined in this study was derived by culling, the demographics of the sample may be representative of the population at large. The sex ratio of males to females in the present study was 1.2:1, deviating significantly from unity. The proactive cull conducted in the UK (1998-2005), during the randomised badger culling trial (RBCT), reported a sex ratio of unity (Jenkins *et al.*, 2008). The higher proportion of males recovered during the present study, may have been a result of differing trapping methodologies. Badgers in Ireland are trapped by reactive culling using stopped restraints, whereas the proactive cull of the RBCT use caged traps. Annual returns from previous badger removal operations in Ireland (1997-2002, excluding 1999), reported yearly sex ratios which did not deviate from unity (O'Boyle, 1998, 1999, 2002,

O'Boyle *et al.*, 2003) . However, the annual returns from 1999 reported a significantly higher proportion of females (O'Boyle, 2000). It is possible that the badger removal operation may interfere with the demographic profile of the Irish badger population, causing it to vary between years. The removal of badgers has been more consistent since 2002-2003; therefore the shift in sex ratio towards male dominance, reported in the present study, may not be a temporal change.

The characteristics of the badgers in the sample examined for the study are those of a population at low to medium density, displaying reduced sociality. When culling is undertaken most restraints are placed at the entrances to active setts, usually at the sett classified as the main sett. However, the majority of badgers in the sample were captured as solitary individuals. It is possible that there were other badgers in the social group which were not trapped, although the trapping was conducted over a two-week period. In addition, setts may have been misclassified, resulting in social groups being mistakenly subdivided. Nevertheless, even if the numbers of solitary individuals were over-estimated, it still indicates that badger groups in Ireland, as seen in the Four Area Study (Griffin *et al.*, 2003, Sleeman *et al.*, 2009), are far smaller than the large group sizes described in studies from the south-west of England (Johnson *et al.*, 2001, Neal & Cheeseman, 1996, Stewart *et al.*, 1997a, Tuyttens *et al.*, 2000b); and more similar to those described in low density areas of Scotland and Continental Europe (Scotland: Kruuk & Parish, 1982, Spain: Revilla & Palomares, 2002, Switzerland: Do Linh San *et al.*, 2007, Poland: Kowalczyk *et al.* 2003).

The Irish badger population is most accurately described as one of low to medium density, similar to those described in Katesbridge in Northern Ireland, Cornwall, Avon and Staffordshire in England (Feore & Montgomery, 1999), Speyside in Scotland (Kruuk & Parish, 1987), Donaña in Spain (Revilla & Palomares, 2002), Saint-Blaise-Cressier-Thielle in Switzerland (Do Linh San *et al.*, 2007) and Bialowieza Primeval Forest in eastern Poland (Kowalczyk *et al.*, 2003). In 1989-90, the badger population in east Offaly was estimated to have a mean group size of 5.8 (range: 1-12) (O'Corry-Crowe *et al.*, 1993), but more recent estimates are 2.3 in Northern Ireland (Feore & Montgomery, 1999) and a mean group size of 2.9 was estimated for the Four Area Study (Sleeman *et*

al., 2009). All of these estimates are far higher than for the present study (mean 1.6; range 1-9), which suggests that badger density may have decreased over the past decades. The badger removal operation conducted by DAFF may have contributed, in part, to this reduction. Alternatively, the results of the present study may have been a biased sample. Bite wounds are indicative of inter- and intra- group aggression, related to territorial defence, mate guarding, sexual competition and protection of off-spring (Cresswell *et al.*, 1992, Delahay *et al.*, 2006b, Neal & Cheeseman, 1996, Woodroffe *et al.*, 1993). Bite wounding was rare within the sample population, with only 0.5% of the sample having evidence of wounds or scarring. This suggests that there is a high degree of tolerance both within and between groups of badgers in Ireland. Discrete territories exist regardless of density level (Feore & Montgomery, 1999), however, behavioural observations have witnessed individuals from adjoining groups foraging close to each other, at boundary areas where their ranges overlapped, without evoking aggression (MacDonald *et al.*, 2002b, Stewart *et al.*, 2002). Intrusions between groups in the present study must occur with limited aggression; it has been suggested that this may be connected to a high degree of relatedness among neighbouring groups (da Silva *et al.*, 1994). Furthermore, intra-group aggression must also be low, this suggests that there may be no dominance hierarchy within this population, consistent with other reports (Kruuk, 1978, MacDonald *et al.*, 2002b), and that reproductive suppression by competing females may be rare.

Neither body length nor femur length were appropriate measures of age classification. The lack of variation obtained between sub-adults (juveniles) and adults may be related to capture methodology. As stopped-restraints where used to catch badgers, only individuals approaching adult size would be caught. Therefore, body length, hind foot length and femur length may show more variation and be more useful in separating sub-adults and adults in studies were badgers were obtained as road casualties or caught in cages. As was proposed in a previous study from the south-west of England (Page *et al.*, 1994), baculum length may be useful in separating sub-adult males from adults. However, extreme caution would be needed in using this as the sole method of aging as there was a high degree of variation between individuals for all age categories except aged adults. Furthermore, there was a continued increase in baculum length through the age categories suggesting that growth does not stop when sexual maturity is achieved.

Body weight and condition showed wide seasonal fluctuations. Both body weight and body condition scores (subcutaneous fat depth and kidney fat index scores) followed similar annual patterns, being relatively low for the first half of the breeding year (March-August), and increasing considerably during the second half of the year to a peak in late autumn and early winter. The patterns were comparable between the sexes. Such increases in body condition and weight are a commonly reported phenomenon in badgers (Kruuk & Parish, 1983, Page *et al.*, 1994) that live in areas where food availability and accessibility is reduced in winter (Roper, 1994). Page *et al.* (1994) presented data for the south-west of England, where males had far greater body weights than females for each month of the year. In the present study, body weight varied little between the two sexes.

Female body weight and condition scores were maximal prior to the periods of implantation, gestation and lactation. High fat reserves are necessary to meet the energy demands of early lactation, as sows remain with their cubs in the immediate post partum period (Delahay *et al.*, 2006a, Woodroffe & MacDonald, 1995a). Females in poor condition in autumn may fail to complete gestation (Delahay *et al.*, 2006a, Woodroffe & MacDonald, 1995a). In the study sample, pregnant females had significantly higher body weights and subcutaneous fat depths than non-pregnant females during the same period. Woodroffe & MacDonald. (1995) detected no effect of gestation on body condition and concluded that the detrimental effects of lactation were greater than gestation. In the present study post-partum females had significantly lower body weight and condition scores than pregnant females during the same period, providing support for Woodroffe's hypothesis.

The peaks in male body weight and condition scores occurred in November-December, prior to resumption of sexual activity. The period was marked by rising testosterone levels, which were maximal in December-January, and increasing testicular weights, which were greatest in February. It is possible that the increases in body weight and condition are necessary for survival, but it is more likely that they are influential in the success of males in intra-sexual competition and in female mate choice.

Disease status was not related to any demographic feature. The location of visible BTB lesions agreed somewhat with previous descriptions (Clifton-Hadley *et al.*, 1993), in both studies lymph nodes associated with the head and lungs had high frequencies. However, the previous study described higher frequencies of lesions in the lungs and kidneys. It is possible that few examples of individuals with lesions in their lungs or kidneys were found in the present study as badgers with generalised BTB were excluded.

4 FEMALE REPRODUCTIVE TRACT MORPHOLOGY

4.1 Introduction

Changes in the basic morphology of the reproductive tract, particularly size and cellular composition, occur during the different stages of the reproductive cycle. This chapter will focus on the morphological changes in the female tract: the length of the uterine horns and the diameter and mass of the ovaries.

Progesterone levels vary during the course of the oestrous cycle. Produced by the corpora lutea, progesterone is the dominant reproductive hormone during the luteal phase of the oestrus cycle, metoestrus and dioestrus. Levels of progesterone are lower during the follicular phase, pro-oestrus and oestrus, due to the absence of functioning corpora lutea. Progesterone is involved in preparation of the reproductive tract for implantation and gestation, and in inhibiting sexual receptivity (Senger, 1997). The cycle of circulating progesterone will be described for the study population.

Uterine length, ovarian diameter, ovarian weight and circulating progesterone were analysed in relation to the demographic factors: month and badger age.

4.2 Results

4.2.1 Uterine Length

4.2.1.1 Description

The lengths of the left and right uterine horns were recorded for each individual. There was a significant difference between left and right uterine lengths (t-test: t=–2.571; d.f.=473; p=0.010), with right uterine lengths being greater than left (Mean uterine horn length: left = 89.49 ± 1.20mm; right: 94.16 ± 1.39mm). Therefore, it was not meaningful to calculate overall mean uterine length, so left and right uterine lengths were analysed separately, providing sample sizes of N=236 and N=239, respectively. Both data sets were normally distributed, allowing for the use of parametric statistical analysis. In examples where compared sub-groups did not share equal variance, non-parametric statistical tests were used.

4.2.1.2 Effect of time of year

Left and right uterine lengths displayed similar patterns throughout the year, with right uterine lengths being consistently higher than left (Figure 4-1). There was a significant influence on both left and right uterine lengths by month (Kruskall Wallis: left uterine horn lengths, χ^2=32.508; d.f.=11; p=0.001; right uterine horn lengths, χ^2=35.444; d.f.=11; p<0.001).

There was a gradual increase in both left and right horn lengths from March until May (Figure 4-1). Length remained relatively constant until January, when there was a sharp decrease towards February. Uterine lengths, for left and right, in February were significantly lower than August and December (Dunn's post hoc test: Left, p<0.05 in both cases; Right p<0.05 in both cases). Right uterine horn lengths in March were also significantly lower than August and December (Dunn's post hoc test: p<0.05 in both cases)

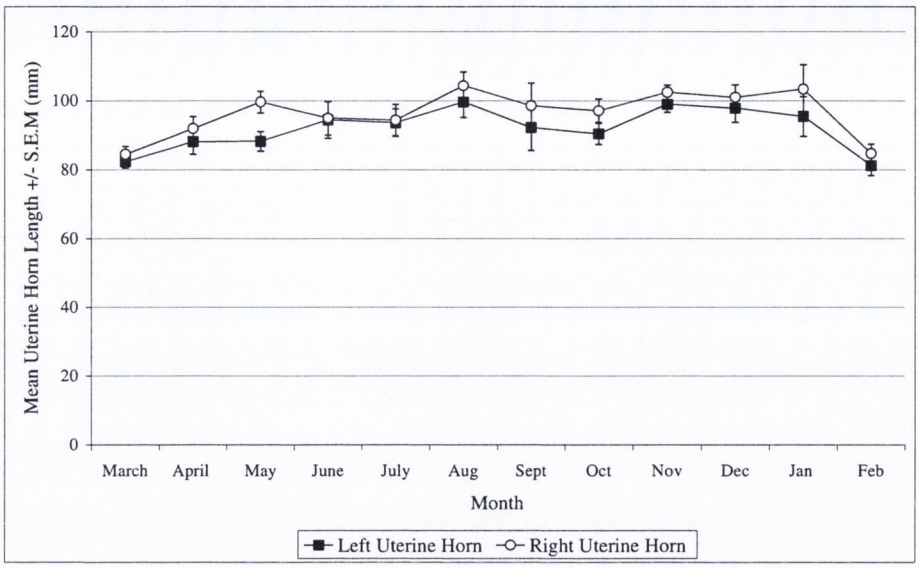

Figure 4-1: Variations in mean left and right uterine lengths by month. Error bars represent standard error of mean.

4.2.1.3 Effect of age

There was a steady increase in length from yearling to aged badgers for both left and right uterine horns, with the right length being consistently longer than the left (Figure 4-2). Left and right uterine horn length varied significantly with age (ANOVA: left uterine horn length, $F=19.806$; d.f.=2; $p<0.001$; right uterine horn lengths, $F=15.125$; d.f.=2; $p<0.001$).

Aged badgers had longer uterine horns (both left and right) than adults and yearlings (Bonferoni post hoc test: right - aged vs. adult, $p<0.01$; $p<0.001$ in all other cases) (Figure 4-2). In addition, adult badgers had longer left and right uterine horns than yearlings (Bonferoni post hoc test: $p<0.05$ in both cases), which had the shortest uterine horns.

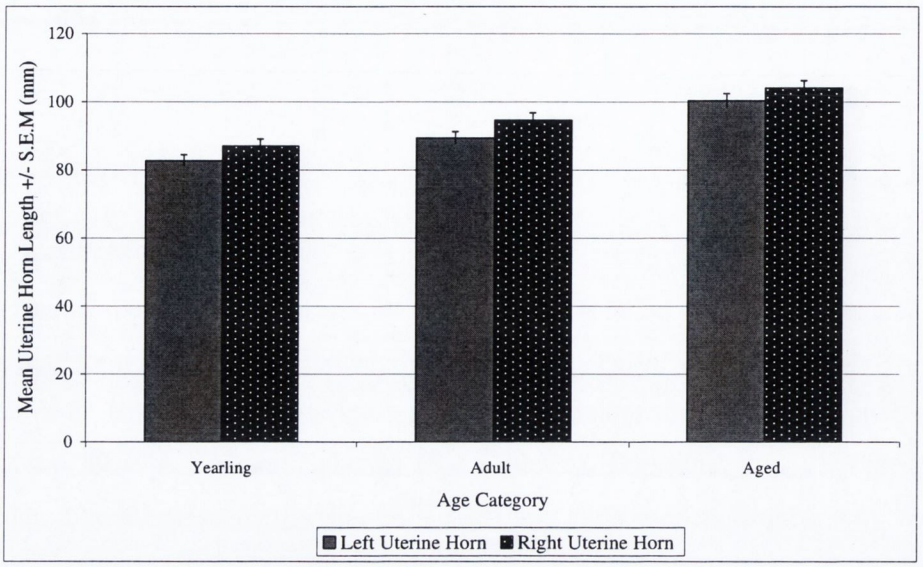

Figure 4-2: Mean left and right uterine horn lengths for the three age categories. Error bars represent standard error of mean.

4.2.2 Ovarian Weight and Diameter

4.2.2.1 Description

Weight and diameter of the left and right ovaries were recorded for each individual. All the data sets were normally distributed, so parametric methods of analysis were used. In examples where compared sub-groups did not share equal variance, non-parametric statistical tests were used.

There was no significant difference between left and right ovarian weights (t-test: t=–0.374; d.f.=514; p=0.709), but there was a significant difference between left and right ovarian diameters (t-test: t=–2.807; d.f.=516; p=0.005). Therefore, it was possible to use the mean weight of the left and right ovaries to represent each individual. As the left and right ovaries did not differ significantly in weight, in those cases where only one ovary weight was recorded for an individual, that is, where ovaries were lost or damaged during the post mortem procedure, the individual was included in subsequent analysis. This provided a sample size of N=258 for mean ovarian weight. The left and right ovarian

diameters were kept separate, providing a sample size of N=230 for left and N=233 for right.

The mean, minimum and maximum values were calculated, separately, for left and right ovarian diameter, for the three age categories (Table 4-1). That the right ovary had a greater diameter than the left ovary was consistent for all age groups. These may have been real differences between left and right, as a lack of symmetry of shape was seen between left and right. It is also possible but less likely that the observed difference may have been due to measurement errors. Generally, the ovaries were irregular in outline and measurement of diameter may not have been uniform. In addition, some distortion of the ovaries may have occurred when measured with the calliper as the ovary is not rigid. Therefore, as ovary weight was a more reliable measurement than diameter, with symmetry between left and right, mean weight was taken as a more accurate indicator of ovary morphology and was used for the following analysis.

Table 4-1 : Descriptive statistics for left and right ovary diameters.

Statistic	Yearling (mm)		Adult (mm)		Aged (mm)	
	Left N=86	Right N=88	Left N=85	Right N=85	Left N=59	Right N=60
Mean	6.92	7.02	6.78	7.27	6.99	7.33
Min	4.23	4.67	3.89	4.05	4.93	3.30
Max	11.08	9.78	8.90	11.03	13.72	14.25

4.2.2.2 Effect of time of year on ovarian weight

Ovarian weight varied significantly with month (Kruskall Wallis: χ^2=63.383; d.f.=11; p<0.001). There were two main peaks, one post partum in April and a second in January-February during gestation (Figure 4-3). A lesser peak also occurred in July. April, July, January and February were months of increased ovarian activity. April was associated with ovulation and fertilisation and January and February were associated with

implantation and gestation. The biological importance of the spike in ovarian related activities in July is unknown and may be an artefact of the data set.

Ovarian weight was high at the beginning of the breeding cycle in March and reached a peak in April (Figure 4-3). Weights in April were significantly higher than the weights in October and November (Dunn's post hoc test: Apr vs. Oct, $p<0.001$; Apr vs. Nov, $p<0.05$). The lesser peak in July was only significantly higher than October (Dunn's post hoc test: $p<0.05$).

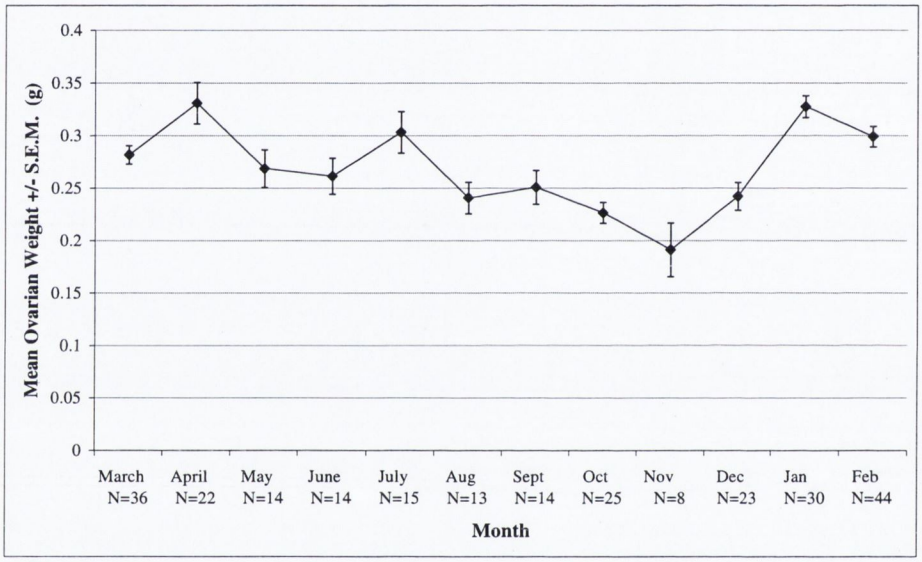

Figure 4-3: Variation in mean ovarian weight by month. Error bars represent standard error of mean.

There was a gradual reduction in ovarian weight from the April peak (disregarding the spike in July) until November when the lowest values were recorded (Figure 4-3). Subsequently, ovarian weight increased to a peak in January-February; ovarian weights in January were significantly higher than the four months: August, October, November and December and February ovarian weights were significantly higher than October and November (Dunn's post hoc test: Jan vs. Oct, $p<0.001$, Jan vs. Dec & Feb vs. Oct, $p<0.01$; $p<0.05$ in all other cases).

4.2.2.3 Effect of age on ovarian weight

All three age categories had similar ovarian weights (Kruskall Wallis: χ^2=4.064; d.f.=2; p=0.131), (Figure 4-4).

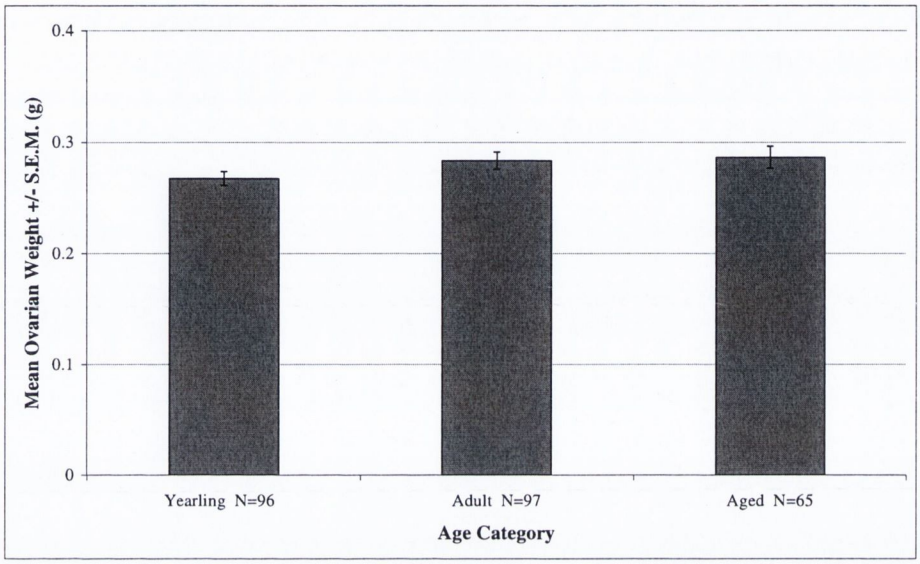

Figure 4-4: Mean ovarian weight for the three age categories. Error bars represent standard error of mean.

4.2.3 Circulating Progesterone

4.2.3.1 Description

The level of circulating progesterone was recorded for each individual. The extreme outliers were removed (cut-off: <–2.341 and >4.095ng/ml, n=11; for criteria see Section 2.5) which provided a sample size of N=198. The distribution of the data set was normalised by natural log transformation, allowing for the use of parametric statistical analysis. Where circulating progesterone levels were correlated with other parameters, the original non-transformed data set (excluding extreme outliers) was used along with non-parametric methods of statistical analysis.

4.2.3.2 Effect of time of year

Progesterone levels gradually increased from low levels in February-April, to the greatest peak in January (Figure 4-5). Implantation and gestation occur during January. There was also a lesser peak in July. The lowest values occurred during February-April; February was associated with the end of gestation and beginning of parturition, making March and April post partum months.

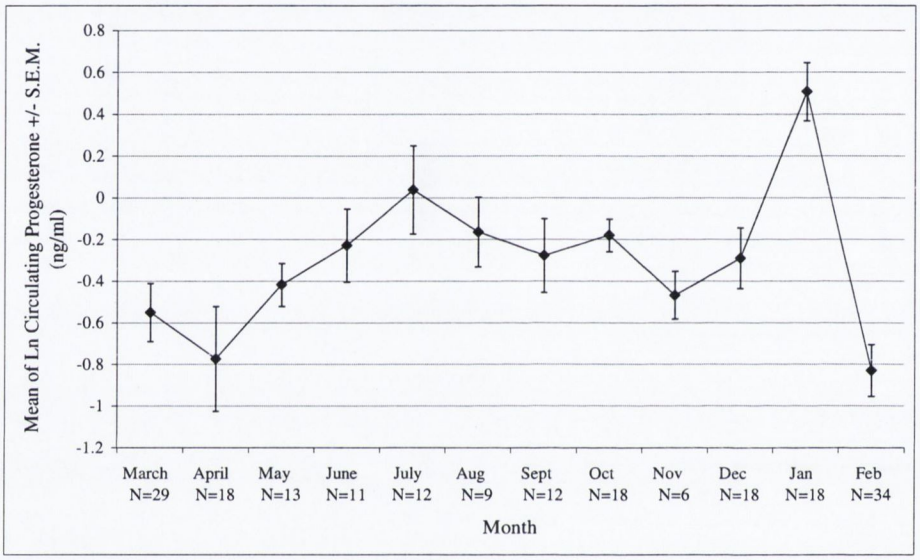

Figure 4-5: Variations in mean circulating progesterone levels by month. Error bars represent standard error of mean.

Circulating progesterone levels varied significantly by month (Kruskall Wallis: χ^2=49.942; d.f.=11; p<0.001). The low peak in July (Figure 4-5) was only significantly higher than the very low values recorded in February (Dunn's post hoc test: p<0.05). The relatively high peak in January was significantly higher than progesterone levels than in February, March and April (Dunn's post hoc test: p<0.001 in all cases).

4.2.3.3 Effect of age

All three age categories had similar progesterone levels (Figure 4-6) and circulating progesterone levels did not vary significantly with age (ANOVA: F=0.023, d.f.=2, p=0.978).

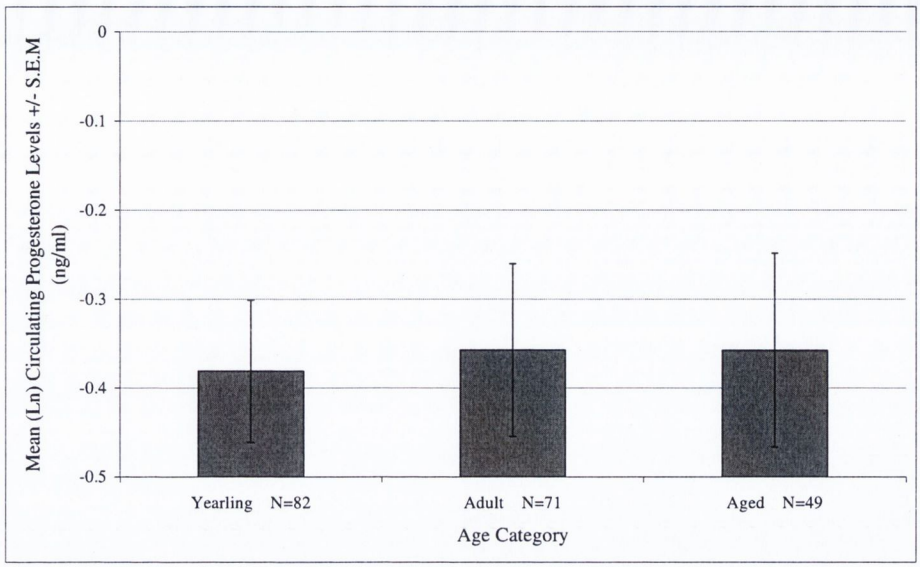

Figure 4-6: Mean circulating progesterone levels for the three age categories. Error bars represent standard error of mean.

4.2.3.4 Relationship of circulating progesterone to ovarian weight

Although there was a slight upward trend in progesterone levels in relation to ovarian weight (Figure 4-7), there was no significant correlation between these two parameters (Spearman's rank-order: r_s=0.061; N=198; p=0.394).

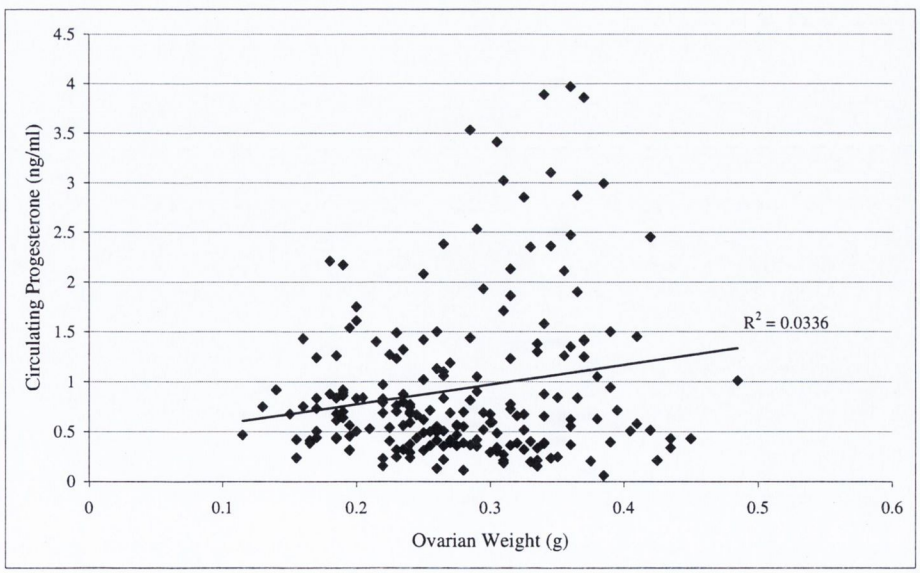

Figure 4-7: Distribution of circulating progesterone levels in relation to ovarian weight for each badger.

4.3 Summary

- Uterine length varied little with time of year. The lowest values occurred in February and March.

- Ovarian weight gradually declined through the breeding year from March to November, being interrupted by two small peaks in April and July. There was a distinct recovery in ovarian weight from December to the greatest peak in values in January.

- Circulating progesterone levels gradually increased during the breeding year from March to the first, relatively low, peak in July. This was followed by a rapid increase from December to the greatest peak in progesterone levels in January. February-April had the lowest levels of circulating progesterone.

- There was no significant association between ovarian weight and circulating progesterone. January and to a lesser extent July had high ovarian weights and high circulating progesterone levels, but February and April had high ovarian weights and low progesterone levels, therefore, ovarian weight is not predictive of progesterone levels.

- Uterine length increased with age but ovarian weight and circulating progesterone levels did not.

4.4 Discussion

Parturition occurs during the months January-March (Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). In the present study the lowest values for uterine length where found within this period (February-March). This reduction may represent a contraction in length following the cubs being born. The appearance of the uterine horns

at post partum is that of increased diameter but reduced length. However, measurements detailing the annual variation in diameter were not recorded.

Implantation occurs during December-January for the majority of females (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981). The onset of implantation is connected to the reactivation of the corpora lutea within the ovaries. During this process the corpora lutea increase in diameter and their associated cells double in size (Canivenc & Bonnin, 1981, Mead, 1981). The morphological changes in the corpora lutea may have contributed to the increase in ovarian weight in January. Ovarian weights were also greatest during April; a month associated with ovulation and fertilisation (Ahlund, 1980, Neal & Harrison, 1958, Paget & Middleton, 1974, Whelan & Hayden, 1993). These two stages in the female reproductive cycle are associated with cellular changes to the ovaries, which may account for the increase in ovarian weight (see Section 5.2.2.3 & Section 5.2.3.6 for relationship of pre-ovulatory follicles and corpora lutea to ovarian weight).

Badgers have been reported to exhibit a biphasic pattern of progesterone secretion; the first rise occurring in July-September and the second in December-January (Bonnin *et al.*, 1978). Comparable results were obtained for the present study. Circulating progesterone levels gradually increased during the breeding year from March to the first, relatively low, peak in July. This was followed by a rapid increase from December to the greatest peak in progesterone levels in January. The first relatively low peak in July is most likely connected to resumption of low levels of luteal activity in the corpora lutea which promotes secretion within the uterine endometrium (Bonnin, 1964). The second maximal peak in January may be associated with resumption of full luteal activation, thought to be necessary for early embryo development, implantation and gestation (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981, Mead, 1981).

February-April had the lowest levels of circulating progesterone, probably for two reasons. Firstly, parturition occurred during February and March (see Section 5.2.5.3); and the corpora lutea of pregnancy are said to regress within three weeks of parturition (Neal & Harrison, 1958). Consequently progesterone levels decline and a new breeding season can begin. Secondly, in agreement with previous studies (Ahlund, 1980, Neal &

Harrison, 1958, Paget & Middleton, 1974) the first ovulations of the year occurred during February-April. Oestrogens are the dominant reproductive hormone during the follicular phase of the oestrous cycle (Mondain-Monval *et al.*, 1980) and following lutenisation (formation of the corpora lutea) the corpora lutea enter a phase of relative inactivity (Canivenc & Bonnin, 1981), which result in low levels of progesterone release (Bonnin *et al.*, 1978)

Ovarian weight was not predictive of progesterone levels. January and to a lesser extent July had high ovarian weights and high circulating progesterone levels, but February and April had high ovarian weights and low progesterone levels. The high ovarian weights and circulating progesterone levels described in January and July can be linked to luteal activity as explained above. The high ovarian weights but low progesterone levels in February and April may be related to the retention of regressing corpora lutea and the increase in large pre-ovulatory follicles and inactive corpora lutea of delay which would contribute to ovarian weight but not progesterone secretion.

5 FEMALE REPRODUCTIVE CYCLE

5.1 Introduction

The female reproductive cycle encompasses the oestrous cycle, fertilisation, gestation, parturition, and post natal care in the form of lactation. This chapter will focus on each stage of the reproductive cycle by identification and analysis of the anatomical and physiological components. The components will be analysed in relation to presence, incidence, annual timing, age of the individual, changes in the reproductive tract, hormone levels and in some cases by comparison with other stages of the cycle.

In addition to the generally recognised stages of the reproductive cycle in mammals, badgers have a period of delayed implantation which can last up to ten months (Perry, 1971) There is little understanding as to how the blastocyst is maintained within the uterus following fertilisation and what triggers implantation. In this chapter the function of the corpora lutea (number and dimension) and the role of circulating progesterone during the process of delayed implantation will be examined and I will postulate as to their importance.

Mammalian females may be monoestrus (one oestrous cycle per year), polyoestrus (oestrous cycles uniformly distributed throughout the year), or seasonally polyoestrous (number of oestrous cycles which occur during a certain season) (Senger, 1997). The incidence and frequency of oestrual females combined with the occurrence of fertilisation

will be examined to determine which of these reproductive strategies is employed by the female badger.

It is important to determine the possible reproductive potential of the Irish badger population and not to rely on data from other countries as variations may occur based on habitat and levels of persecution. The reproductive potential and reproductive success of the study population was investigated by comparing the present potential for pregnancy with evidence of past pregnancies, by contrasting the performance of the study population at each stage of the cycle and by investigation of possibility of inter-group reproductive suppression.

In polyoestrous females the oestrous cycle occurs repeatedly, providing females with continued opportunities to conceive. The cycle consists of two phases, the follicular phase and a longer luteal phase. The follicular phase covers pro-oestrus and oestrus; and commences following a decline in progesterone levels which is instigated by regression of the corpora lutea (luteolysis) from the previous oestrous cycle or from pregnancy. Throughout both stages, pro-oestrus and oestrus, the dominant hormone is oestradiol. During pro-oestrus, oestradiol is involved in the stimulation and development of pre-ovulatory follicles, the release of a mature ovum (ovulation), the preparation of the reproductive tract for ovulation and mating; and during oestrus it induces behavioural alterations and causes further physiological changes to the reproductive tract. It is during oestrus that females become sexually receptive and will copulate (Senger, 1997, Sternberg, 1997). The follicular phase was identified in sampled females by the presence of pre-ovulatory follicles on the ovary and physiological changes to the endometrium (the membrane lining the uterus) and the vaginal epithelium.

The luteal phase of the oestrous cycle, which covers metoestrus and dioestrus, begins with ovulation and the formation of a corpus luteum at the site of the newly ovulated follicle. The corpus luteum acts as an endocrine gland during metoestrus and dioestrus. Metoestrous is associated with the formation of fully functional corpora lutea. Until the corpora lutea are fully functional progesterone levels remain low. Dioestrus is the longest stage of the cycle and is the period when the corpus luteum is producing high levels of

progesterone. High progesterone levels stimulate the preparation of the uterus; making it a suitable environment for maintenance of the blastocyst, early embryo development and implantation. Progesterone is involved in a negative feedback loop which prevents the female from becoming sexually receptive until ovulation re-occurs. Although follicles may continue to grow they will regress before ovulation can occur and behavioural symptoms characteristic of oestrus will be suppressed. The end of dioestrus is marked by luteolysis, and a consequent rapid decline in progesterone levels; hence the negative feedback loop is disrupted allowing the oestrus cycle to proceed (Senger, 1997, Sternberg, 1997). The luteal phase of the cycle was categorised in sampled females by the presence of corpora lutea, increases in progesterone levels and changes to the reproductive tract.

Anoestrus is the time when a female does not display regular oestrous cycles and occurs during pregnancy, immediately post partum, may be a seasonal characteristic of the species (seasonal anoestrus), and may be induced by stress or pathology (Senger, 1997, Sternberg, 1997). Anoestrous was detected in study females by examination of the uterine epithelium using histology and the condition of the ovaries.

Following copulation, a single spermatozoon fuses with the oocyte, thus forming a zygote. The zygote endures a series of cleavage divisions (mitotic divisions) resulting in a 2-celled embryo. There are subsequent divisions into 4-, 8-, and 16-celled embryos. At this stage there is no increase in the size of the embryo; instead the dividing cells become progressively smaller. Once the embryo has reached a stage where the cells are no longer readily countable it is referred to as a morula. The cells within the morula segregate into inner and outer cells layers due to compaction. The outer cells adhere tightly to one another (tight junctions) which alters the permeability of the cells; fluid accumulates within the embryo forming a cavity. Once the cavity becomes distinct (blastocoele) the embryo is referred to as a blastocyst (Dellmann & Eurell, 1998, Senger, 1997, Sternberg, 1997). For the study, females with blastocysts present were categorised as having been fertilised.

Continued mitotic divisions of the blastocyst and a build up of fluid in the blastocoele, increases the pressure within the blastocyst. Concurrently, the outer (trophoblastic) cells

produce proteolytic enzymes which weaken the outer layer (zona pellucida) of the embryo. These two factors contribute to the easy rupture of the embryo, which is followed by the gentle release of the cells from within the blastocyst. The conceptus begins to grow more rapidly and develops a set of extra-embryonic membranes, which are essential for implantation within the uterine wall. At this stage of gestation high levels of progesterone are essential for the development of the embryo, for increased secretion by the endometrial glands and for implantation. Pregnancy recognition by the female is essential as it prevents luteolysis, and allows for progesterone concentrations to remain high and for the foetus to continue to grow and develop (Senger, 1997, Sternberg, 1997). The presence of foetuses within the uterus classified females, from the study population, as gestating. Information collected about the foetuses was used to determine pregnancy rate, fecundity, implantation dates and parturition dates

The extra-embryonic membranes of the embryo contribute to the formation of the placenta which provides a semi-permanent link between the female and the foetus. In badgers, the placenta is zonary and is made up of three areas: the first is responsible for exchange of nutrients and metabolic by-products and is formed centrally around the foetus; the second is highly pigmented due to small haematomas and encompasses the central region; and finally, the third which is transparent may function in the absorption of materials directly from the uterine lumen. It is the second pigmented zone that results in placental scarring. Placental scars were identified in the study females as an indication of past pregnancies. One of the polypeptide hormones produced by the placenta is placental lactogen. This hormone promotes foetal growth and development of the mammary glands (Senger, 1997, Sternberg, 1997). The status of mammary gland development was examined in females from the study population using histology, and females were categorised according to the level of development.

During parturition progesterone dominance is replaced by oestrogen and the corpora lutea of pregnancy undergoes luteolysis, thus removing the negative feedback loop. The rapid decline in progesterone results in the transition from anoestrus of pregnancy into a new oestrous cycle. However, despite the high levels of oestradiol, females remain in anoestrous for a short time following parturition to facilitate uterine involution, which is

essential for subsequent conception and avoidance of infection (Senger, 1997, Sternberg, 1997).

5.2 Results

5.2.1 Oestrous Cycle – Incidence

5.2.1.1 Description

The incidence of each stage in the oestrous cycle was determined by histological examination of the endometrium (N=246) and the vaginal epithelium (N=222). From examination of the endometrium it was possible to differentiate between pro-oestrus, oestrus and anoestrus but not metoestrus and dioestrus, so when reporting on the state of the uterus the latter were combined. In the examination of the vaginal epithelium a distinction could not be made between metoestrus, dioestrus or anoestrus, so when reporting on the state of the vaginal epithelium these three stages were combined, providing three states: pro-oestrus, oestrus and metoestrus/dioestrus/anoestrus.

In 90.54% of cases, classification of the two tissues into stages of the oestrous cycle was identical. For the other cases, placement of individuals into stages of the oestrous cycle from examination of the vaginal epithelium was either one (9.01%) or two (0.45%) stages behind the stage identified from examination the uterine endometrium. This suggests that there may be a delay in progression through the stages of the oestrus cycle in the histology of the vaginal epithelium compared to the endometrium. Alternatively, identification errors may have occurred.

In addition, the thickness of the vaginal epithelium was measured (N=218). The distribution of this data set was not normal; therefore, non-parametric methods of analysis were used.

5.2.1.2 Oestrous cycle timing according to the endometrium

Using the histological condition of the endometrium as a guide, females displayed two periods of oestrus during the year, one occurring early in the year between January and April and the second between June and November (Figure 5-1). Within these periods, the proportion of females in oestrus varied widely. The highest proportions of oestrual females occurred in February-March (37.21%-41.18%) and November (37.5%). June and August also had high proportions of females in oestrus (33.33%). July, October and January had the lowest proportions of oestrual females (3.33%-7.69%).

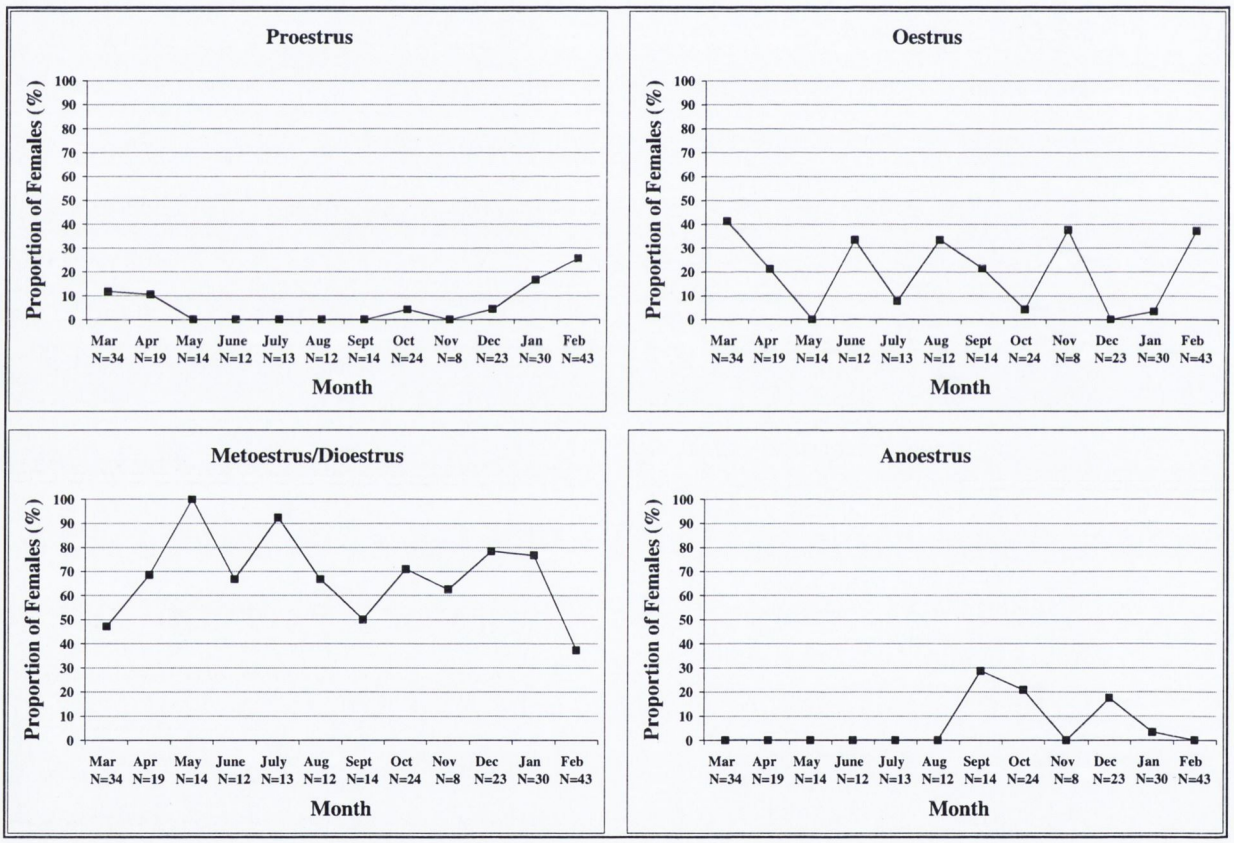

Figure 5-1: Proportion of females, as a percentage of the study population, displaying the various stages of the oestrus cycle based on histology of the endometrium.

Pro-oestrus occurred in October and December to April (Figure 5-1). Proportions of females showing evidence of pro-oestrus were initially low, but increased to a peak in

February (the post-partum period); there was a subsequent reduction in proportions. Females in metoestrus/dioestrus occurred throughout the year, being highest in May (100%) and lowest in February (37.21%). Anoestrus was only seen during a five month period, with highest proportions in September, gradually declining through the following four months, October to January. It is worth noting that 13 out of 14 (92.86%) of the anoestrual females were yearlings. Although all had pre-ovulatory follicles, only one had corpora lutea and two had blastocysts despite not having any corpora lutea. Therefore, the majority may not have been fully sexually active. The remaining anoestrual adult female was obtained during September and had no corpora lutea or blastocysts present.

5.2.1.3 Oestrous cycle timing according to the vaginal epithelium

From interpretation of the histology of the vaginal epithelium, it was established that oestrual females were present during each month of the year (Figure 5-2). The proportion of females in oestrus peaked in February-March, fluctuating at relatively high proportions throughout the following months (disregarding July and October); finally declining to low proportions in December-January. The highest proportions of oestrual females were seen in February-March (45.24%-45.16%), with May, June and November, also having high proportions (37.5%-38.46%). The lowest proportions of oestrual females occurred during December-January (4.55%-3.57%).

Pro-oestrus females were present during two periods of the year, December-April and July-August (Figure 5-2). Proportions increased from zero in November to a peak in February (23.81%). The second period of pro-oestrus during July-August had lower proportions of females (8.33%). Females displaying metoestrus/dioestrus/anoestrus were present throughout the year, being highest during the seven month period, July-January, (66.67%-90.91%) and lowest in February (30.95%).

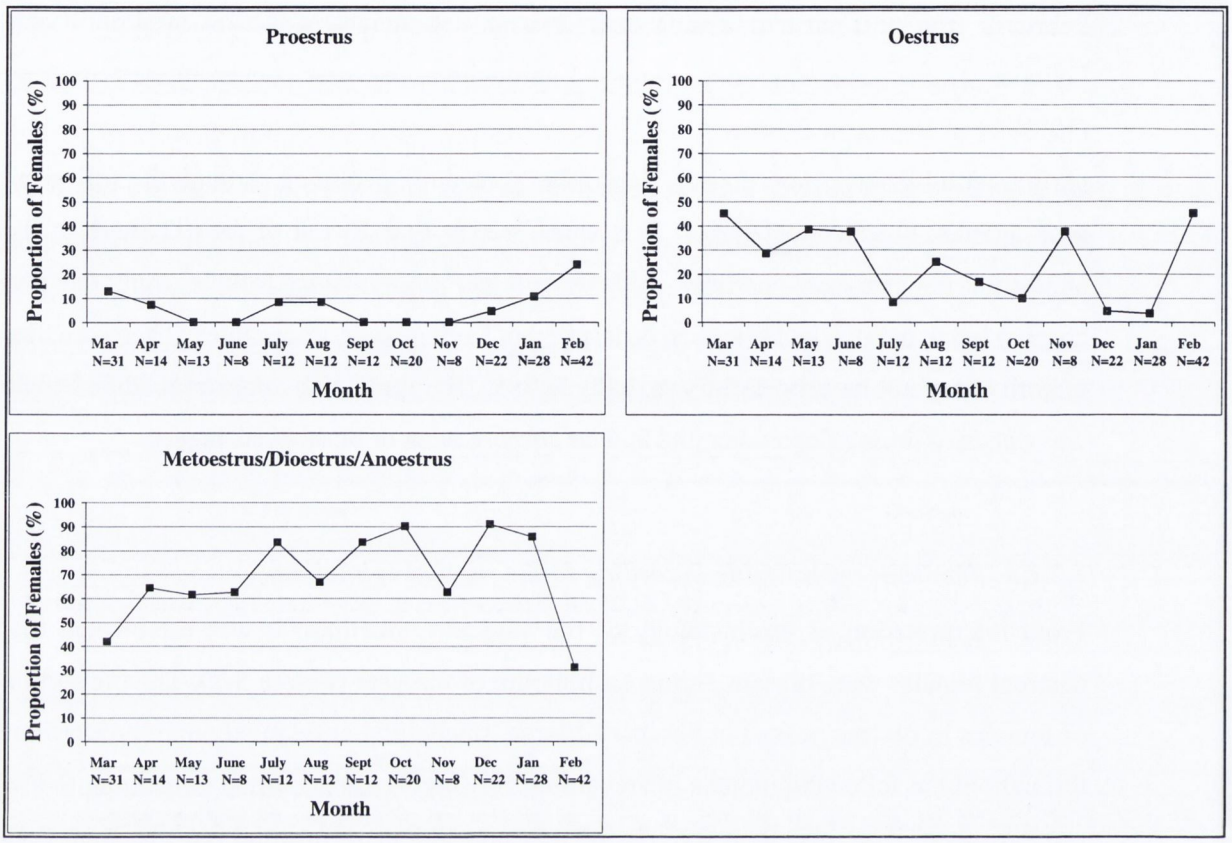

Figure 5-2: Proportion of females, as a percentage of the study population, displaying the various stages of the oestrus cycle based on histology of the vaginal epithelium.

5.2.1.4 Vaginal epithelium thickness related to time of year

The vaginal epithelium was thickest in February; thickness decreased in April and remained reasonably constant until December (Figure 5-3). There was a significant effect of month on epithelium thickness (Kruskall Wallis: χ^2=70.753; d.f.=11; $p<0.001$). The peak in thickness in February was significantly greater than the epithelium thickness of seven months of the year, April-May, July-October and December (Dunn's post hoc test: Feb vs. Apr & Oct, $p<0.01$; $p<0.001$ in all other cases).

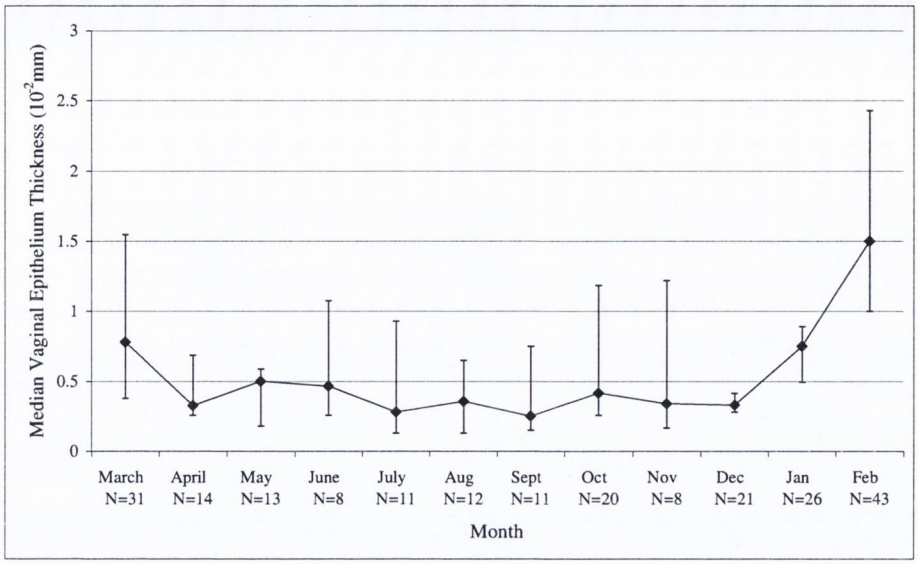

Figure 5-3: Variations in the median thickness of the vaginal epithelium by month. Error bars represent inter-quartile range.

5.2.1.5 Vaginal epithelium thickness related to the stages in the oestrous cycle

There was a peak in vaginal epithelium thickness during oestrus (Figure 5-4) with both pro-oestrus and metoestrus/dioestrus/anoestrus being associated with lower epithelium thicknesses and metoestrus/dioestrus/anoestrus having the lowest thicknesses. Epithelium thickness during oestrus showed the highest animal to animal variation of the three stages.

Epithelium thickness varied significantly with the oestrous cycle (Kruskall Wallis: χ^2=87.414; d.f.=2; p<0.001). Oestrus was associated with the highest epithelium thicknesses being significantly higher than the two other stages and epithelium thickness during pro-oestrus was significantly higher than metoestrus/dioestrus/anoestrus (Dunn's post hoc test: pro-oestrus vs. /metoestrus/dioestrus/anoestrus, p<0.05; oestrus vs. pro-oestrus, p<0.01; oestrus vs. metoestrus/dioestrus/anoestrus, p<0.001).

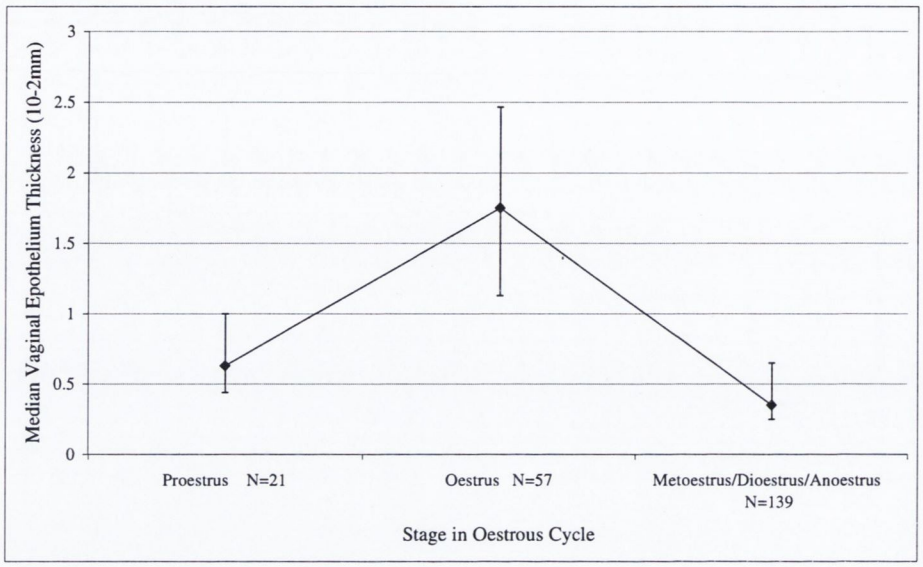

Figure 5-4: Variations in median thickness of the vaginal epithelium by stage in oestrous cycle. Error bars represent the inter-quartile range.

5.2.1.6 Effect of the oestrus cycle on progesterone levels

Circulating progesterone levels increased with progression through the oestrous cycle (classified according to condition of the uterine endometrium) to peak during metoestrus/dioestrus (Figure 5-5). There was a subsequent decline in progesterone levels during anoestrus. Progesterone levels varied significantly with the different stages of the oestrous cycle (ANOVA: $F=7.703$; d.f.=3; $p<0.001$). The increase in progesterone levels during metoestrus/dioestrus was significantly higher than progesterone levels during proestrus and oestrus but not anoestrus (Bonferroni post hoc test: $p<0.01$ in both cases).

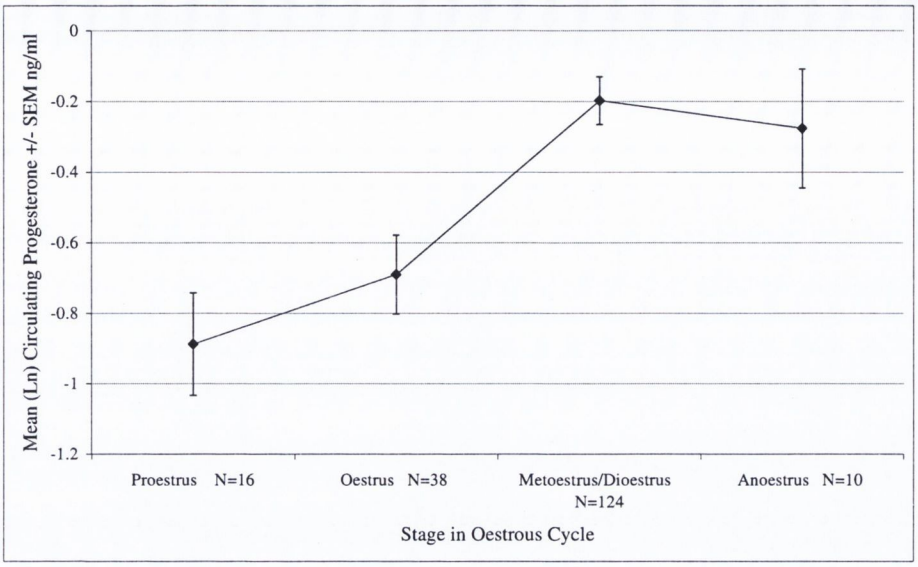

Figure 5-5: Mean circulating progesterone levels for the stages of the oestrous cycle. Error bars represent standard error of mean.

5.2.2 Oestrous Cycle – Follicular Phase

5.2.2.1 Description

Secondary and tertiary follicles were counted on the ovaries. Not all females had secondary and/or tertiary follicles, therefore the sample sizes for females with secondary follicles (N=244) differed from those with tertiary follicles (N=221). The distribution of both data sets was not normal; therefore non-parametric methods of analysis were used.

There were no significant differences in the numbers of secondary or tertiary follicles between left and right ovaries (Mann Whitney, secondary follicles: Z=–0.806; N=259,256; p=0.420; Mann Whitney, tertiary follicles: Z=–1.384; N=259,256; p=0.166). Therefore, numbers of secondary follicles on the left and right ovaries were combined for each individual, as were the data for tertiary follicles.

5.2.2.2 Effect of time of year

Secondary and tertiary follicle numbers followed similar trends throughout the year (Figure 5-6); with the numbers of secondary follicles showing greater variation than tertiary follicle numbers. The peak in secondary follicle number occurred in January, but numbers were also high in February and March. Correspondingly, tertiary follicles were highest in February, but this peak was less well defined than that for the secondary follicles. There was a significant effect of month on both secondary follicle and tertiary follicle numbers (Kruskall Wallis: secondary follicles, χ^2=65.345; d.f.=11; p<0.001; tertiary follicles, χ^2=25.244; d.f.=11; p=0.008).

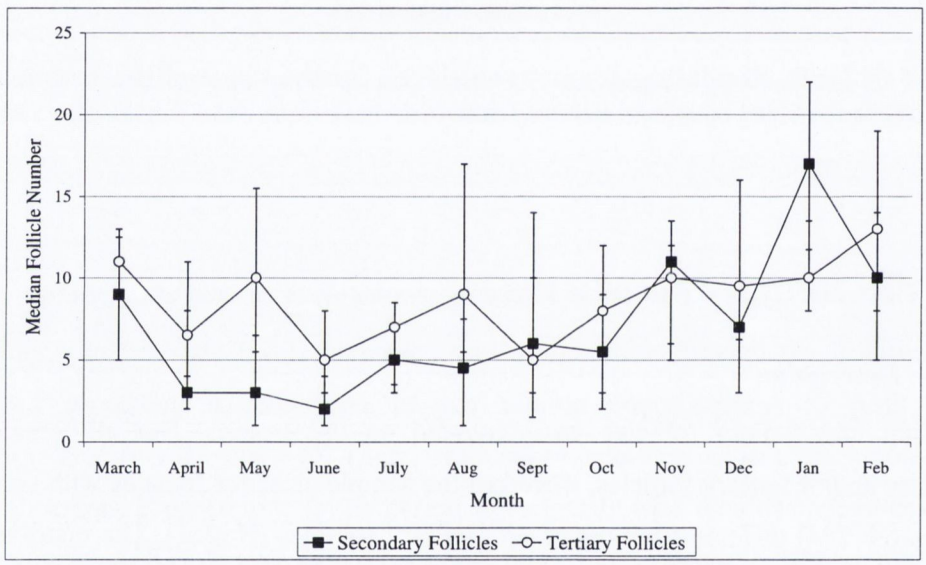

Figure 5-6: Variations in median secondary and tertiary follicle numbers by month. Error bars represent the inter-quartile range.

There was a decline in secondary follicle numbers from March until June (Figure 5-6), with June having significantly lower numbers than March, January and February (Dunn's post hoc test: June vs. Mar, p<0.05; June vs. Jan, p<0.001; June vs. Feb, p<0.01). From June secondary follicle number increased to a peak in January, with this month having significantly higher numbers than June but also April, May, August and October (Dunn's post hoc test: Jan vs. Aug, p<0.01; p<0.001 in all other cases). Secondary follicles declined again in February, to a level similar to March.

The number of tertiary follicles fluctuated moderately throughout the year with the lowest numbers in September and a gradual increase to an overall peak in February (Figure 5-6). Dunn's post hoc test did not detect any significant differences.

5.2.2.3 Effect of age

Tertiary follicle numbers were similar for all three age categories with aged badgers having the highest secondary follicle numbers (Figure 5-7). However, there was no significant variation in secondary or tertiary follicle numbers with age (Kruskall Wallis: secondary follicles, χ^2=0.249; d.f.=2; p=0.883; tertiary follicles, χ^2=1.842; d.f.=2; p=0.398).

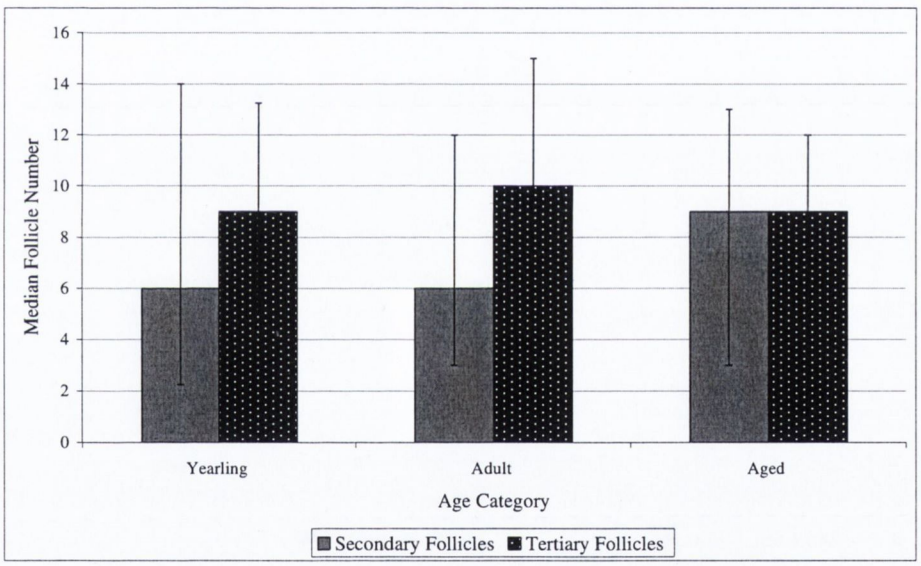

Figure 5-7: Median secondary and tertiary follicle numbers for the three age categories. Error bars represent the inter-quartile range.

5.2.2.4 Relationship of secondary and tertiary follicles to ovarian weight

Although variations in both secondary and tertiary follicle numbers were correlated with ovarian weight, only a small proportion of the weight changes were related to the number of follicles (Figure 5-8). There was a significant, positive correlation between secondary

follicle number and ovarian weight (Spearman rank-order correlation: r_s=0.322; N=230; p<0.001), however, the R^2 value was very low (R^2=0.0938). There was also a significant, but lesser, positive correlation between ovarian weight and tertiary follicle number (Spearman rank-order correlation: r_s=0.208; N=234; p=0.001) with a lower R^2 value being obtained (R^2=0.0547).

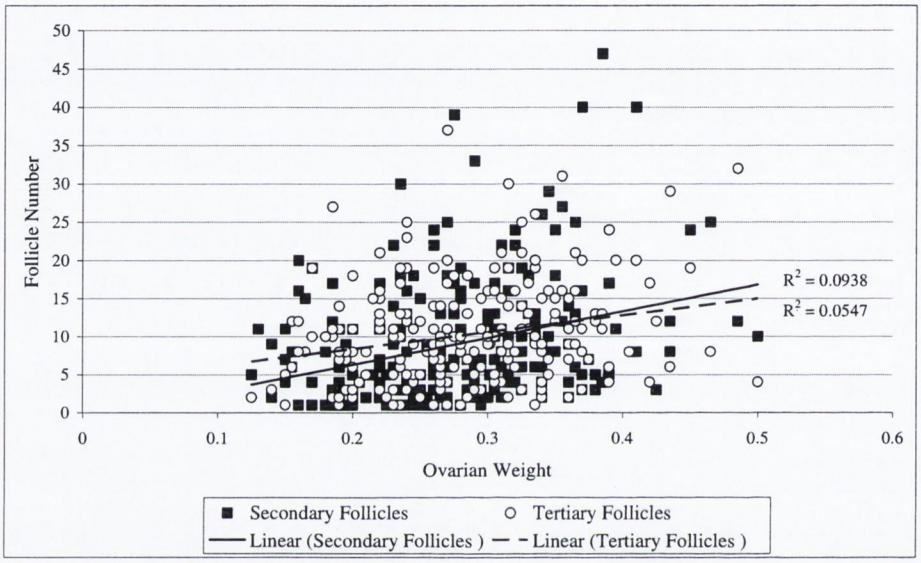

Figure 5-8: Distribution of secondary and tertiary follicle numbers in relation to ovarian weight for each badger.

5.2.2.5 Relationship of secondary and tertiary follicles to levels of progesterone

There was no correlation between increasing progesterone levels and increasing secondary follicle numbers (Spearman rank-order correlation: r_s=0.001, N=187; p=0.993) (Figure 5-9). Correspondingly, there was no correlation between decreasing progesterone levels and increasing tertiary follicle numbers (Spearman rank-order correlation: r_s=–0.129, N=169; p=0.095).

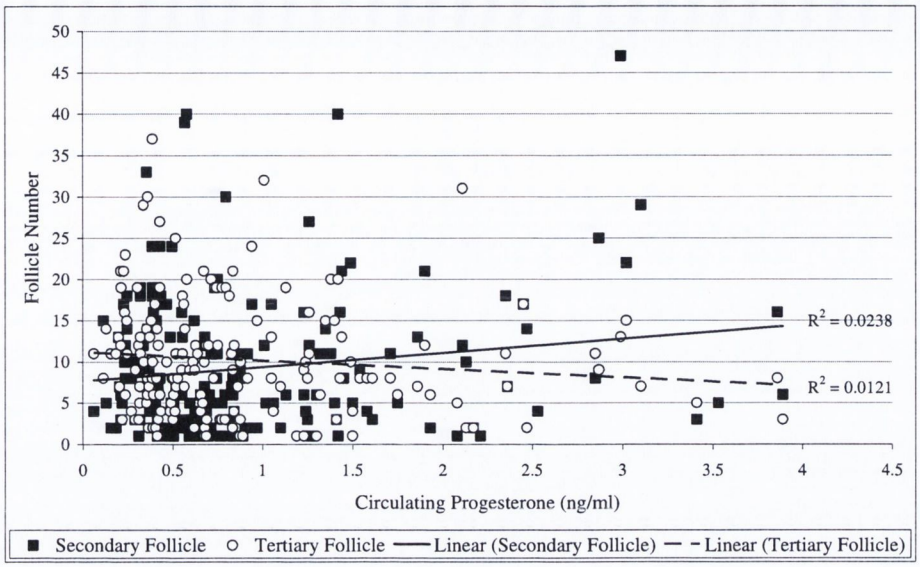

Figure 5-9: Distribution of secondary and tertiary follicles related to levels of circulating progesterone for each badger.

5.2.3 Oestrous Cycle - Luteal Phase

5.2.3.1 Description

The number and dimensions of the corpora lutea on both ovaries were recorded. As there was no significant difference in corpus luteum numbers for left and right ovaries (t-test: t=–0.660; d.f.=502; p=0.510), data pooling provided a total corpus luteum number for each individual. Those individuals with no corpora lutea were excluded from the analysis leaving 204 data points for corpus luteum number and 721 for corpus luteum surface area. The distributions of the two data sets were normal allowing for the use of parametric statistical analysis. In examples where variance was not equal among sub-groups, non-parametric statistical tests were used.

5.2.3.2 Ovulation rate of study population

The proportion of females with corpora lutea showed an annual biphasic distribution (Figure 5-10). The first phase occurred between March and August and the second between September and February. February was associated with the lowest proportion of

females to have corpora lutea present. This month is when the number of pregnant females declines as the number of post partum females increases. During the first phase, there was an increase in the proportion of females possessing corpora lutea from March to May, following which the proportion remained at around 90%.

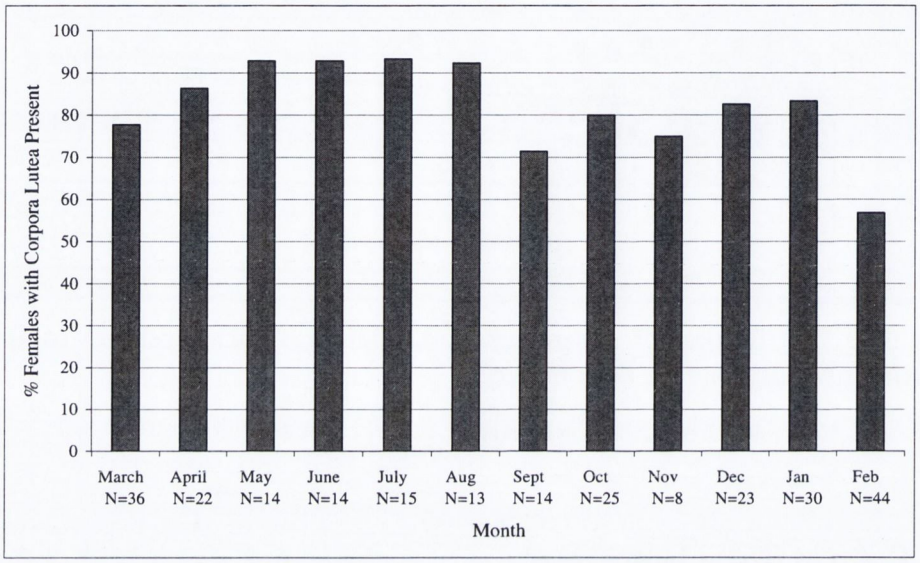

Figure 5-10: Proportion of females, as a percentage of the study population, with corpora lutea present on the ovary.

The second phase, which began in September, started with a gradual increase in the proportion of females with corpora lutea and ended with a steep drop in February (Figure 5-10). The highest proportions within this phase occurred between October and January. The mean of the highest proportions during the second phase was 80%, which was lower than the mean of the highest proportions during the first phase, May-August, which exceeded 90%.

5.2.3.3 Effect of time of year

The mean number of corpora lutea showed a relatively consistent increase during the breeding year; but corpora lutea surface area declined to November, rising to its greatest areas in January (Figure 5-11). This suggests that corpora lutea were continually recruited throughout the breeding year to a peak in November-February. Concurrently, with the

increase in number there was a decrease in the average surface area until November; surface area then increased to the peak in January. December-February was associated with implantation and gestation.

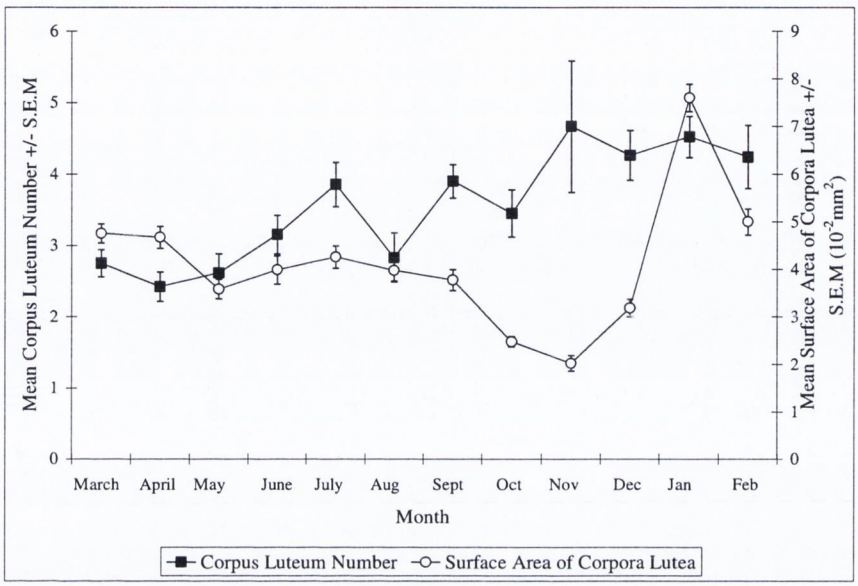

Figure 5-11: Variation in mean number and surface area of corpora lutea by month. Error bars represent standard error of mean.

The effect of month on corpus luteum number was significant (Kruskall Wallis: χ^2=53.326; d.f.=11; p<0.001). There was a gradual increase in mean corpus luteum number from the post-partum period (March-April) to the period of implantation and gestation (December-February) (Figure 5-11). Corpus luteum numbers were significantly higher in December-January compared to March-May and February was significantly higher than April (Dunn's post hoc test: Dec vs. Mar & May and Feb vs. Apr, p<0.05; Dec vs. Apr and Jan vs. Mar & May, p<0.01; Jan vs. Apr, p<0.001).

Mean surface area of the corpora lutea decreased gradually from March to November, followed by a steep increase in January (Figure 5-11). The effect of month on corpus luteum surface area was statistically significant (Kruskall Wallis: χ^2=237.030; d.f.=11; p<0.001). Corpora lutea surface areas in October-December, prior to implantation, were significantly smaller than the begining of the breeding season, February-April (Dunn's

post hoc test: Feb vs. Apr, $p<0.01$; $p<0.001$ in all other cases). October and November, which had the lowest surface areas, were also significantly lower than June-September and May-September, respectively (Dunn's post hoc test: Oct vs. Sept and Nov vs May $p<0.05$; Oct & Nov vs. July, $p<0.001$; $p<0.05$ in all other cases). The peak in corpora lutea surface area in January was significantly greater than all the months of the year, excluding July (Dunn's post hoc test: Jan vs. Apr, $p<0.01$; $p<0.001$ in all other cases).

5.2.3.4 Effect of age

Although corpus luteum number decreased with age (Figure 5-12) the difference was not statistically significant (ANOVA: $F=0.774$; d.f.=2; $p=0.462$).

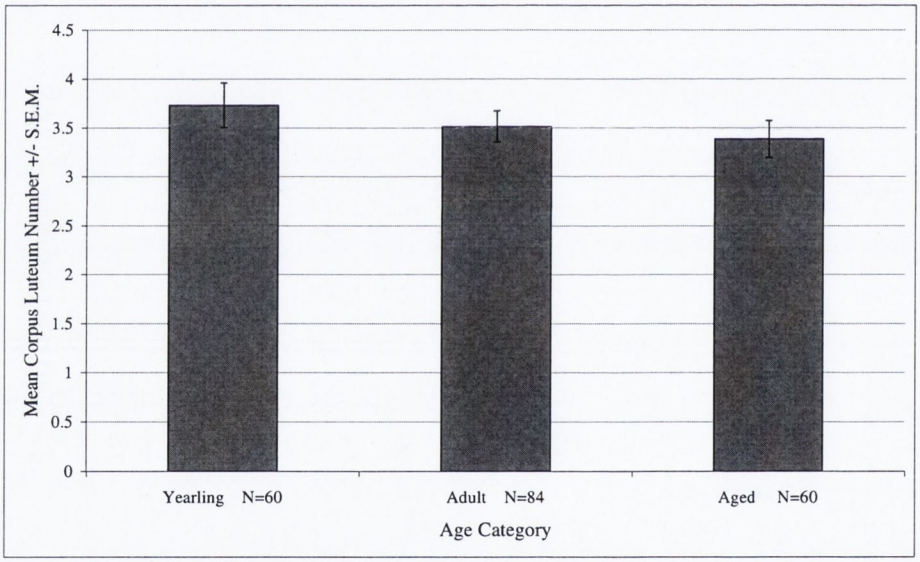

Figure 5-12: Mean corpus luteum numbers for the three age categories. Error bars represent standard error of mean.

Corpus luteum surface area was significantly affected by age (Figure 5-13) (ANOVA: $F=6.237$; d.f.=2; $p=0.002$). There was little difference between yearlings and adult badgers, but aged badgers had significantly lower corpus luteum surface areas than yearlings and adult badgers (Bonferroni post hoc test: aged vs. yearling, $p<0.05$; aged vs. adult, $p<0.01$).

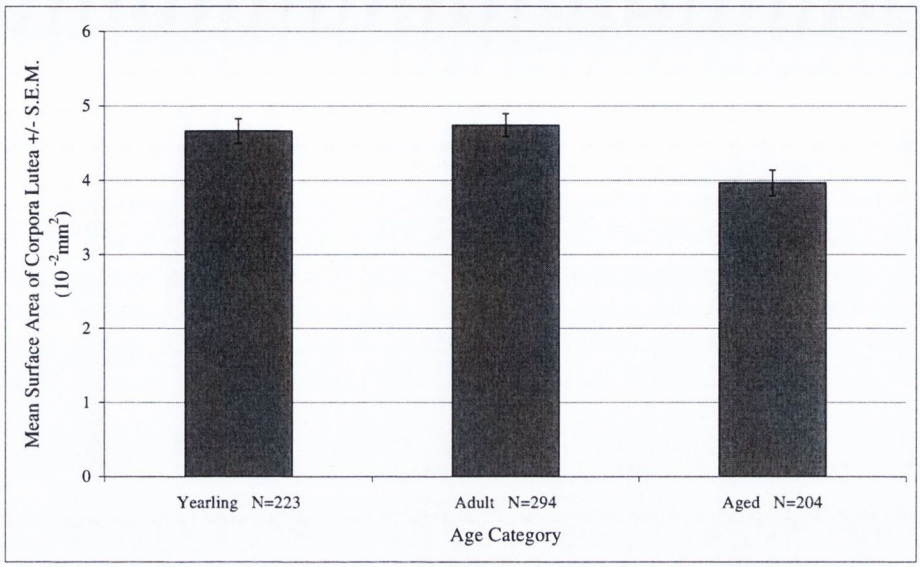

Figure 5-13: Mean corpus luteum surface areas for the three age categories. Error bars represent standard error of mean.

5.2.3.5 Variation in corpus luteum number and dimension in relation to reproductive state

Five reproductive states of the uterus were defined: blastocysts absent, blastocysts present, not pregnant (foetuses absent), pregnant (foetuses present), and post partum (involution of the uterus). The five states were restricted to specific times of the year: absence and presence of blastocysts were restricted to March - December, not pregnant and pregnant restricted to January - February, and the post partum period to January - February. The reproductive cycle was assumed to progress in time through the first four states to the last. The relationship of these five states on the two parameters, corpus luteum number and corpus luteum surface area, was assessed.

There was a significant overall effect of reproductive state on corpus luteum number (Kruskall Wallis: χ^2=29.000 d.f.=4; p<0.001) and surface area (Kruskall Wallis: χ^2=152.224 d.f.=4; p<0.001) (Figure 5-14). There was a gradual increase in corpus luteum number with progression through the reproductive states, with pregnant females having the highest numbers and post partum females the lowest. There was no significant difference in the corpus luteum number of females with blastocysts present and those with blastocysts absent. Corpus luteum number in pregnant females was significantly greater

than those in any other reproductive state, excluding females who were not pregnant in the January – February period (Dunn's post hoc test: foetuses present vs. blastocysts absent: $p<0.001$; $p<0.05$ in all other cases).

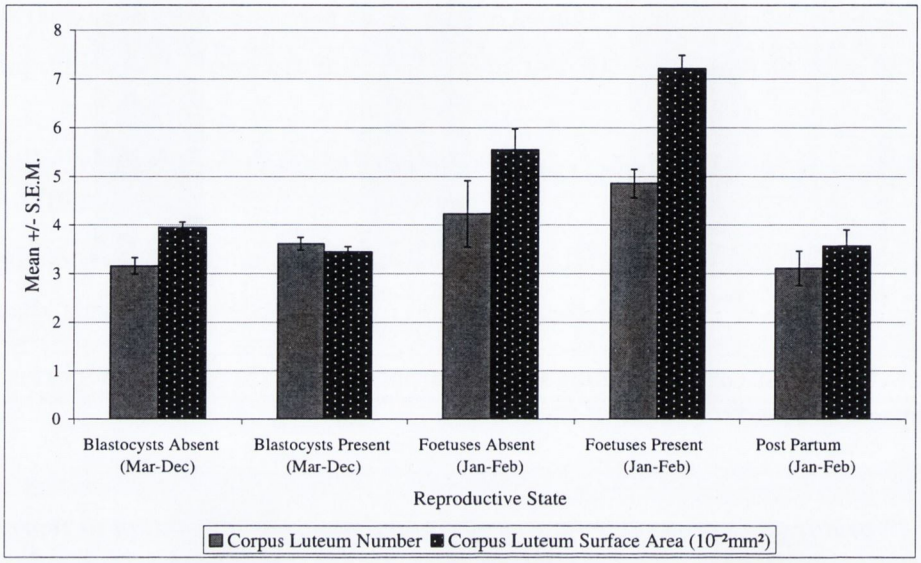

Figure 5-14: The relationship of the mean number of corpora lutea and the mean surface area of corpora lutea in yearlings, adult and aged badgers to reproductive status of the uterus: presence of blastocysts, foetuses and post partum. Error bars represent standard error of mean.

The surface area of corpora lutea increased with progression through the reproductive states (Figure 5-14) and was greatest when females were pregnant and least when blastocysts were present and in the post partum state. Females who were pregnant in January – February had significantly greater corpora lutea surface areas than those not pregnant (Dunn's post hoc test: $p<0.01$) and both of these groups were significantly higher than females with blastocysts absent or present (Dunn's post hoc test: foetuses absent vs. blastocysts absent: $p<0.05$; $p<0.001$ in all other cases). Only females with foetuses present had significantly higher corpus luteum surface areas than post partum females (Dunn's post hoc test: $p<0.001$)

5.2.3.6 Relationship of corpora lutea and ovarian weight

There was a non-significant positive correlation between corpus luteum number and ovarian weight (Spearman's rank-order correlation: r_s=0.112; N=205; p=0.110) (Figure 5-15), and a significant positive correlation between corpus luteum surface area and

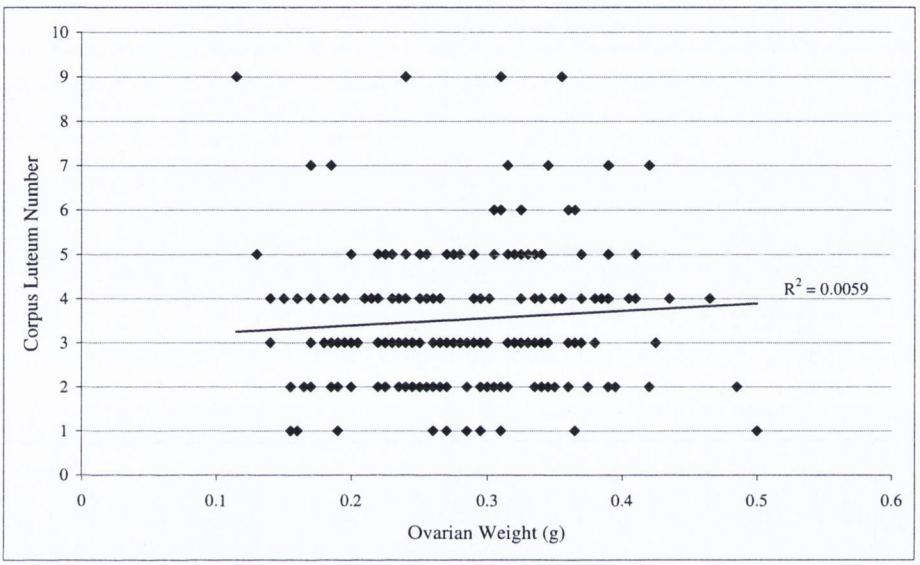

Figure 5-15: Distribution of corpus luteum number in relation to ovarian weight for each badger.

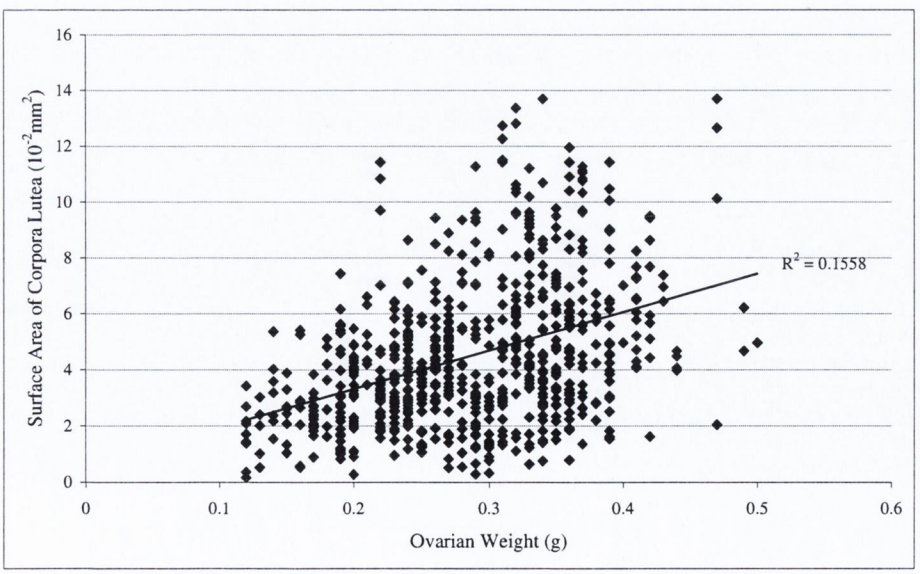

Figure 5-16: Distribution of corpus luteum surface area in relation to ovarian weight for each badger.

ovarian weight (Pearson's correlation: r=0.395; N=721; p<0.001) (Figure 5-16). The latter correlation had an R^2=0.1558, indicating that nearly a sixth of the variation in ovarian weight was associated with changes in surface area.

5.2.3.7 Relationship of corpora lutea to circulating progesterone levels

Circulating progesterone levels were higher for females with corpora lutea present (Figure 5-17). The presence of corpora lutea had a significant effect on progesterone levels (t-test: t=–2.809; d.f.=196; p=0.005).

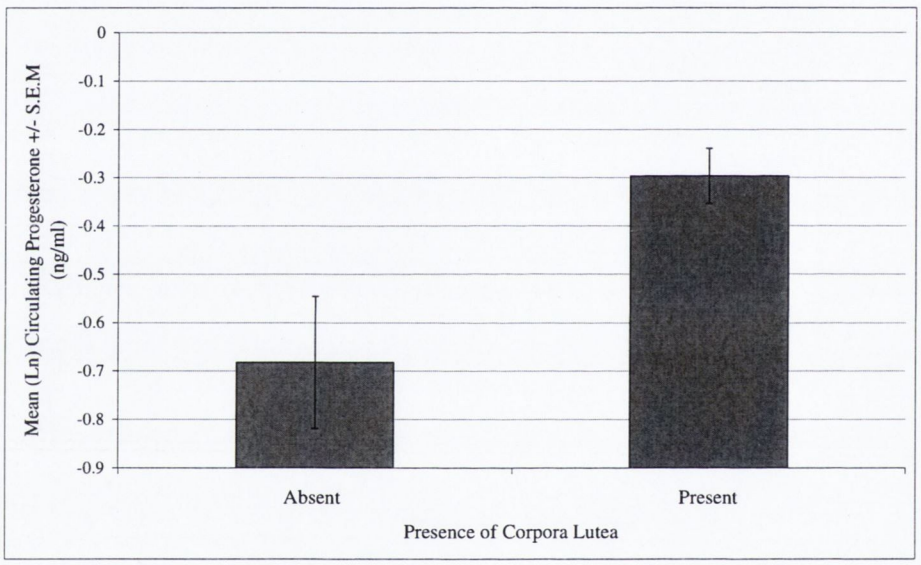

Figure 5-17: Variations in mean circulating progesterone levels by the presence of corpora lutea. Error bars represent standard error of mean.

Increasing circulating progesterone level was associated with both increased corpus luteum number (Figure 5-18) and surface area (Figure 5-19). There was a significant positive correlation between corpus luteum number and circulating progesterone (Spearman rank-order correlation: r_s=0.247; N=162; p=0.002) (Figure 5-18), and, corpus luteum surface area and circulating progesterone (Spearman rank-order correlation: r_s=0.260; N=580; p<0.001) (Figure 5-19). The latter correlation had a R^2 value (R^2=0.1663) indicating that a sixth of the variation in circulating progesterone levels was

associated with changes in corpus luteum surface area. Correspondingly, the R^2 value obtained for the correlation between corpus luteum number and circulating progesterone was low (R^2=0.062), suggesting little association.

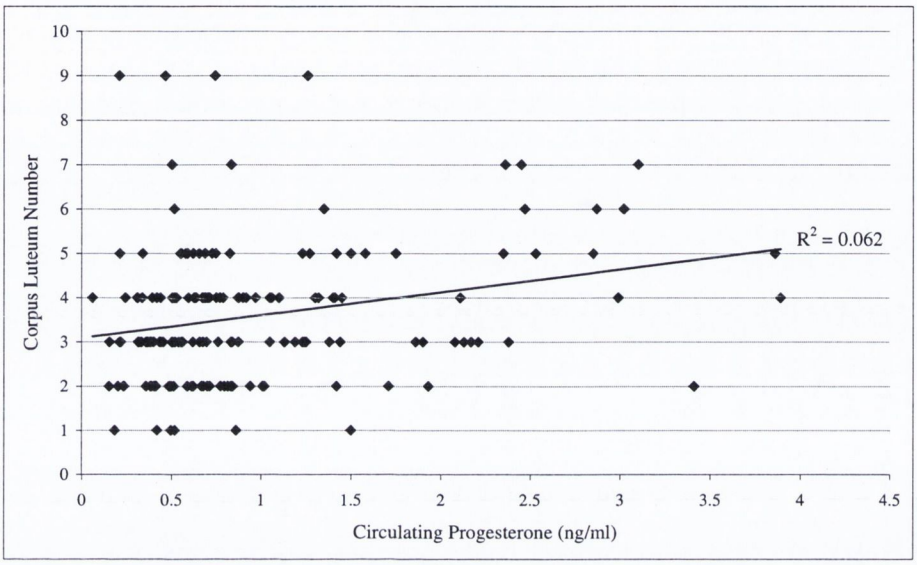

Figure 5-18: Distribution of corpus luteum number in relation to the levels of circulating progesterone for each badger.

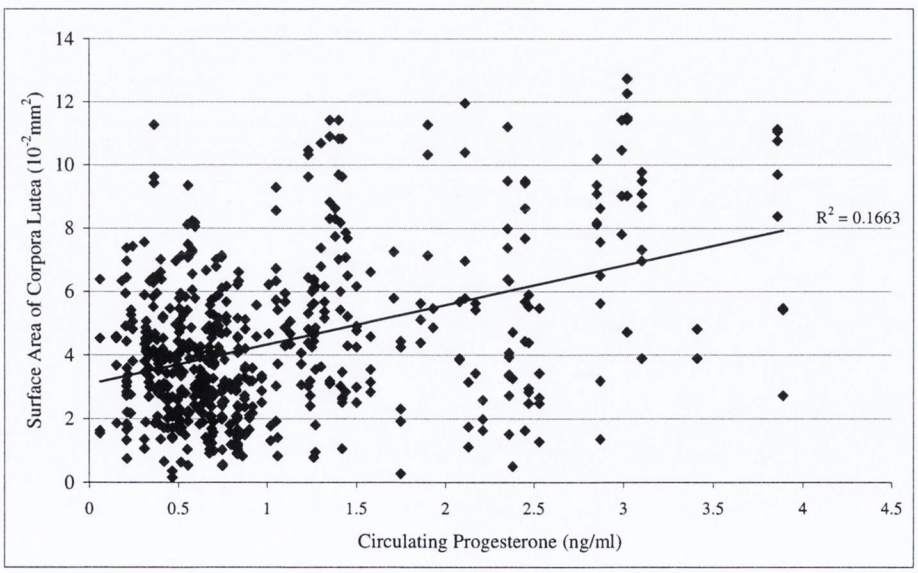

Figure 5-19: Distribution of corpus luteum surface area related to the levels of circulating progesterone for each badger.

5.2.4 Fertilisation

5.2.4.1 Description

The number of blastocyts and the diameter of each blastocyst was recorded for each individual. There was no significant difference in the number of blastocysts collected from the left versus the right uterine horn (Mann Whitney: Z=–0.45; N=172,172; p=0.964). The number of blastocyts for each female was the sum of those present in the left and right uterine horns.

Blastocysts were first detected in March and were last detected in December (10 months). Only individuals with blastocysts present were analysed providing a sample size of N=76 for blastocyst number. One hundred and fifty one blastocysts were collected and provided data on blastocyst diameter. The two data sets were not normally distributed and non-parametric methods of statistical analysis were used.

5.2.4.2 Fertilisation rate of study population

Blastocysts were first observed in March. The proportion of females with blastocysts increased until October and thereafter the proportion remained reasonably constant (Figure 5-20). The proportion remained between 35% and 50% for the four months between April and July, and then increased to 60 - 65% from August to December, excluding September.

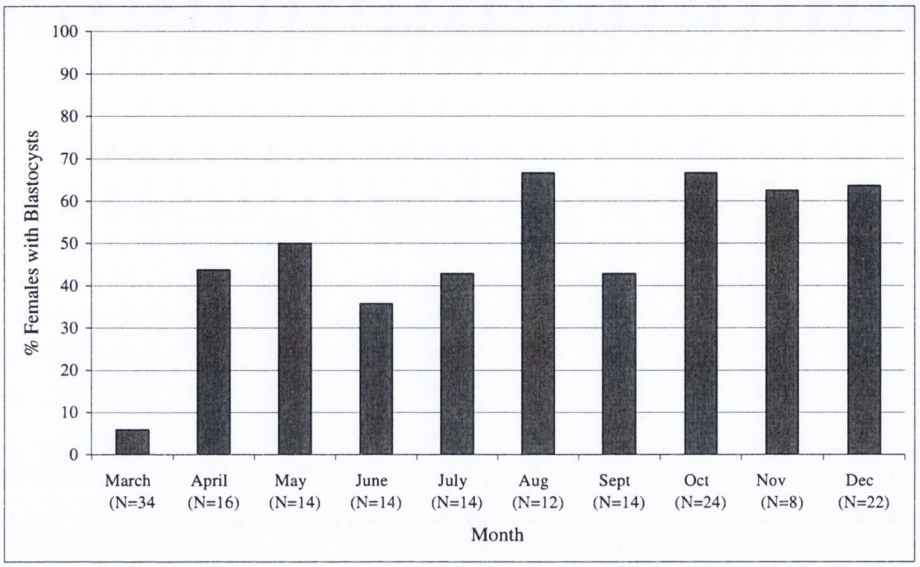

Figure 5-20: Proportion of females, as a percentage of the study population, with blastocysts present in the uterus.

5.2.4.3 Effect of Time of Year

Blastocyst number fluctuated throughout the breeding year to a peak in October-November, which was followed by a decline in December, the period of implantation (Figure 5-21). Blastocyst number varied significantly with month (Kruskall Wallis: χ^2=21.477; d.f.=9; p=0.011). Numbers during October were significantly higher than numbers in May (Dunn's post hoc test: Oct vs. May, p<0.01).

Blastocyst diameter increased with progression through the months of the year (Figure 5-22). There was a significant positive correlation between blastocyst diameter and month (Spearman rank-order correlation: r_s=0.704; N=151; p<0.001). The R^2 value of 0.57, suggests that nearly 60% of the increase in blastocyst diameter was related to time of year.

Blastocyst diameter varied significantly during the year (Kruskall Wallis: χ^2=88.396 d.f.=9; p<0.001) (Figure 5-22). Both the monthly median and range of blastocyst diameters increased slowly from March to August, followed by a rapid increase in September and a levelling out to December. Diameters in the last 3 months of the year

were significantly higher than diameters in the five months from March to July. (Dunn's post test: Oct vs. Mar and Dec vs. Mar & July, $p<0.05$; Oct vs. July, Nov vs. Mar, and Dec vs. May & June, $p<0.01$; $p<0.001$ in all other cases).

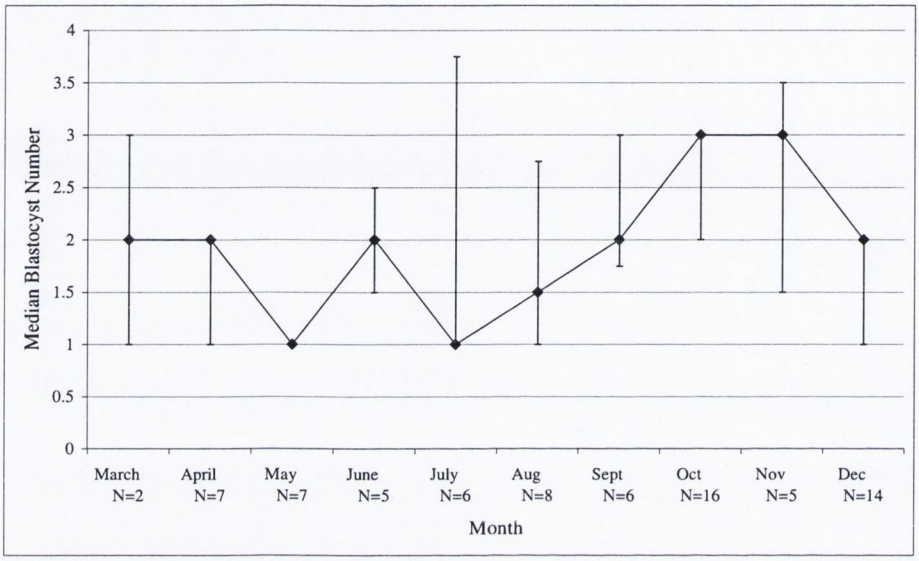

Figure 5-21: Variation in median blastocyst number by season. Error bars represent the inter-quartile range.

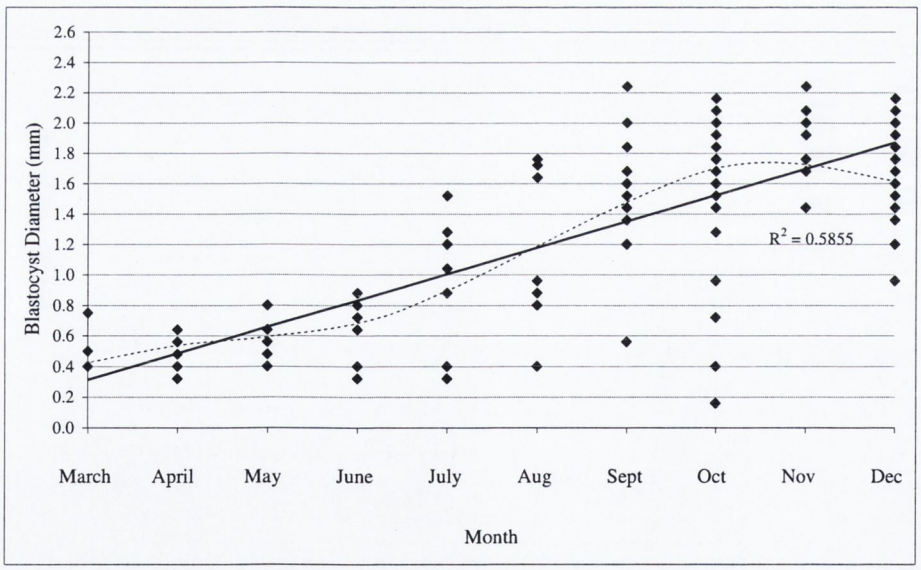

Figure 5-22: Distribution of blastocyst diameters in relation to month including line of best fit; each point represents a single blastocyst. Median for each month represented by ----.

Between September and December, there were blastocysts with diameters indicative of the earlier months (Figure 5-22). Assuming that there is an increasing median diameter from the earlier months until implantation (that is with the age of the blastocyst), the presence of these blastocysts with smaller diameters could be interpreted as indicating a process of continual ovulation and fertilisation throughout the year. Females belonging to this category were either: yearlings where the number of corpora lutea corresponded to the number blastocysts (2% of fertilised females in Sept-Dec, N=41), adults that had excess corpora lutea (5% of fertilised females in Sept-Dec, N=41) or adults that had blastocysts of two distinct sizes and had a corresponding number of corpora lutea compared to number of blastocysts (7% of fertilised females in Sept-Dec, N=41). Furthermore, 18.42% (N=76) of females with blastocysts showed histological evidence (based on proliferation of the endometrium and vaginal cornification) of being in oestrus.

5.2.4.4 Effect of Age

The same median number of blastocysts was observed in all three age categories (Figure 5-23) and there were no significant differences (Kruskall Wallis: χ^2=3.263; d.f.=2; p=0.196).

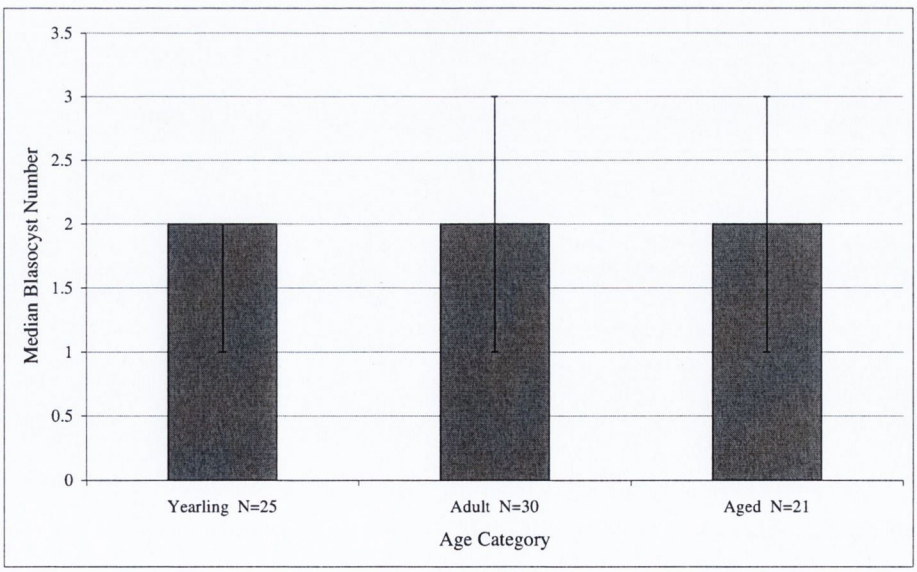

Figure 5-23: Median blastocyst numbers for the three age categories. Error bars represent the inter-quartile range.

There was a gradual increase in blastocyst diameter from yearlings through to aged badgers (Figure 5-24). Blastocyst diameter varied significantly with age (Kruskall Wallis: χ^2=9.115 d.f.=2; p=0.010). Aged badgers had significantly greater blastocyst diameters than yearling and adult badgers (Dunn's post hoc test: p<0.05 in both cases). However, the majority of aged badgers were obtained during the last four months of the calendar year (37/50), which are associated with greater blastocyst diameters. When only the last four months were compared age did not have a significant effect (Kruskall Wallis: χ^2=4.787 d.f.=2; p=0.091). It is unlikely that age had a true effect on blastocyst diameter.

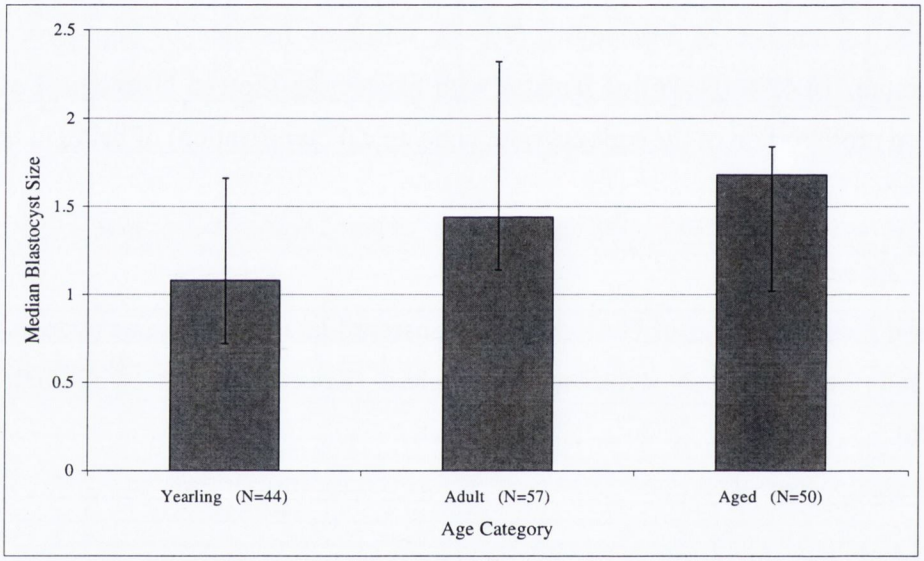

Figure 5-24: Median blastocyst diameter for the three age categories. Error bars represent the inter-quartile range.

5.2.4.5 Relationship of blastocyst number and diameter to corpora lutea

There was a significant positive correlation between blastocyst diameter and corpus luteum number (Spearman rank-order correlation: r_s=0.282; N=151; p<0.001) (Figure 5-26) but the positive correlation between blastocyst number and corpus luteum number was not significant (Spearman rank-order correlation: r_s=0.168; N=76; p=0.147) (Figure 5-25). However, for the former correlation the R^2 value obtained was very low (R^2 = 0.0669) suggesting that corpus luteum number had little influence on blastocyst diameter.

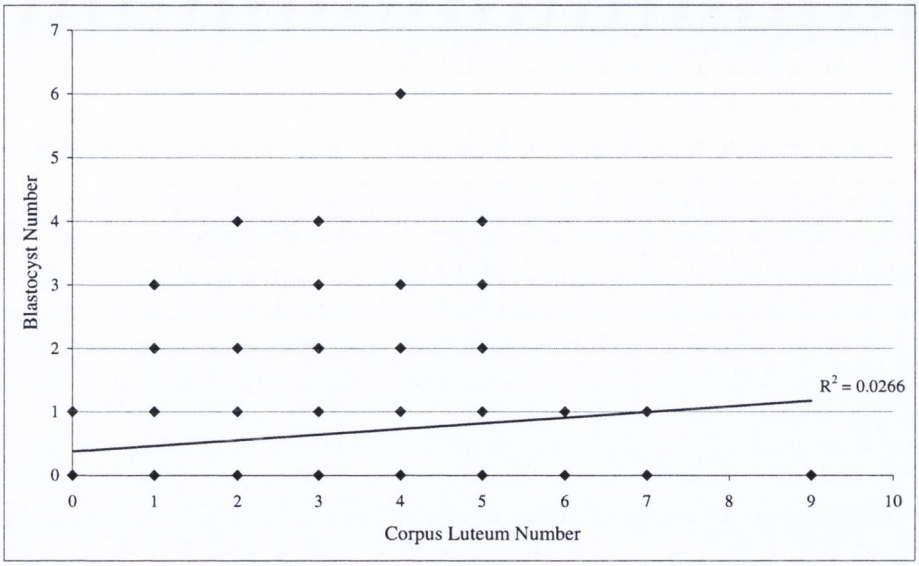

Figure 5-25: Distribution of blastocyst number in relation to corpus luteum number for each badger.

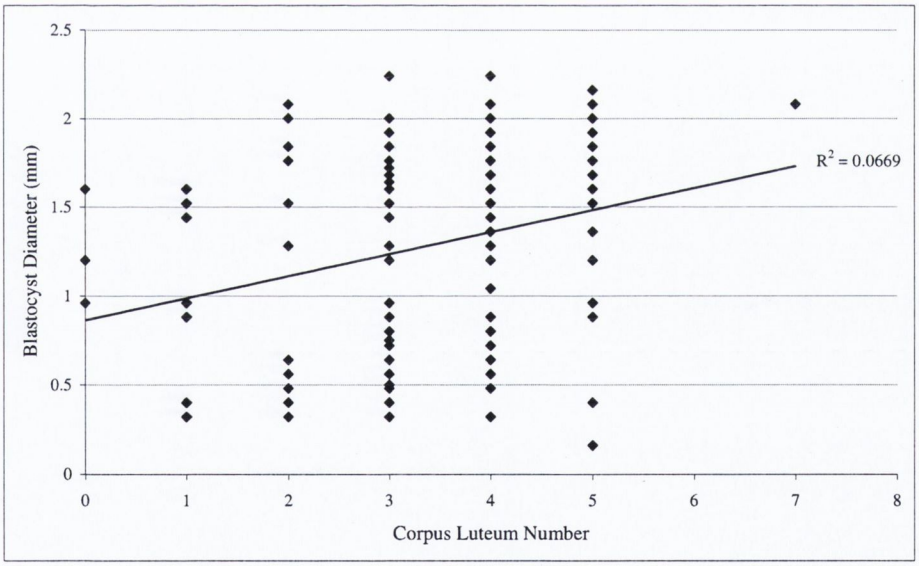

Figure 5-26: Distribution of blastocyst diameter in relation to corpus luteum number for each badger.

Corpus luteum number was in excess of blastocyst number for 68.42% of females possessing blastocysts (N=76). Therefore, a comparison was made between mean

blastocyst number and mean corpus luteum surface area. There was a negative association between blastocyst diameter and corpus luteum surface area (

Figure **5-27**): the correlation was significant (Spearman rank-order correlation: r_s=–0.539; N=70; p<0.001). In addition, the R^2 value obtained was reasonably high (R^2 = 0.2453), which suggests that one quarter of the variation in blastocyst diameter was negatively related to corpora lutea surface area.

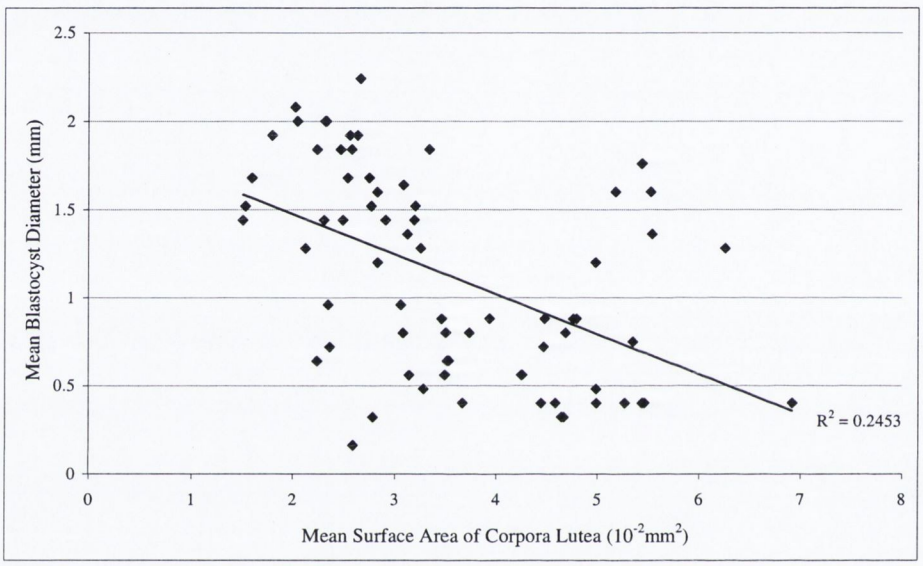

Figure 5-27: Distribution of mean blastocyst diameter in relation to mean corpus luteum surface area for each badger.

5.2.4.6 Relationship of blastocyst number and diameter to levels of circulating progesterone

Circulating progesterone was not significantly related to variation in blastocyst number (Figure 5-28) or blastocyst diameter (Figure 5-29). There was a significant positive correlation between blastocyst diameter and circulating progesterone levels (Spearman rank-order correlation: r_s=0.211; N=123; p=0.019), but the R^2 value was very low (R^2 = 0.0072) suggesting that these two factors were not influencing each other. Correspondingly, the positive correlation between blastocyst number and circulating

progesterone levels was not significant (Spearman rank-order correlation: r_s=0.122, N=62; p=0.344).

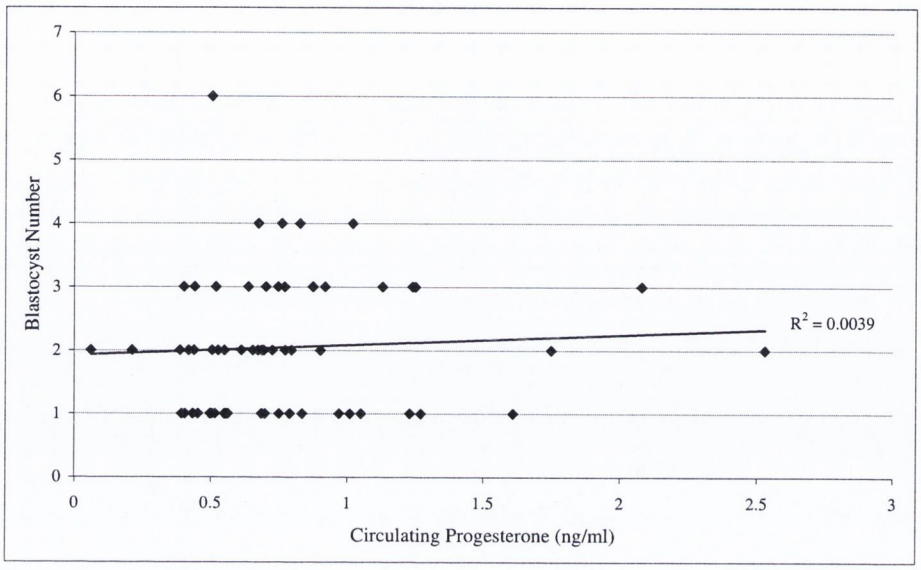

Figure 5-28: Distribution of blastocyst number in relation to circulating progesterone levels for each badger.

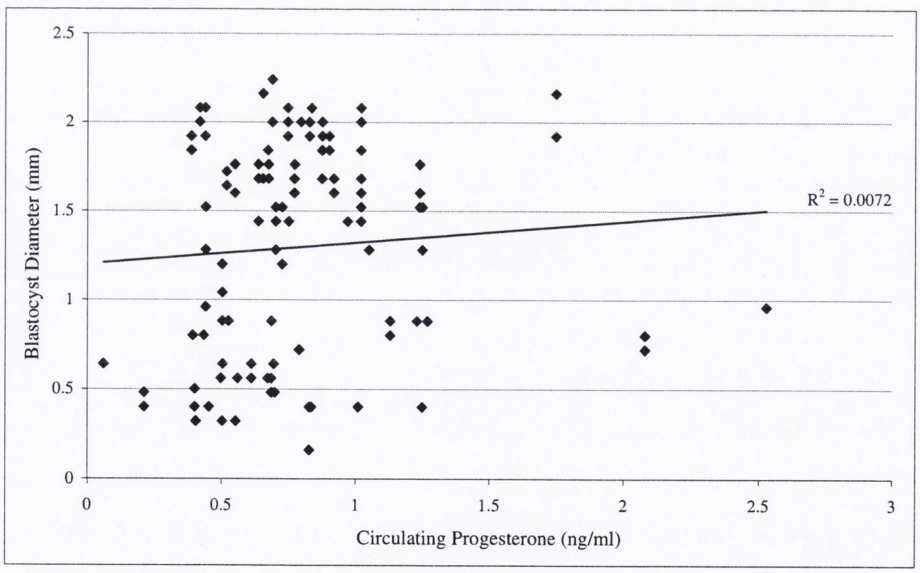

Figure 5-29: Distribution of blastocyst diameter in relation to circulating progesterone levels for each badger.

5.2.5 Pregnancy

5.2.5.1 Description

Pregnancy was defined as the period when foetuses were detected in the uterus. Foetus number, length and weight were recorded for each pregnant female. There was no significant difference between the number of foetuses collected from the left and right uterine horns (t-test: t=0.000; d.f.=146; p=1.000).

Foetuses were only found during January and February. The pregnancy rate was calculated from all females examined during January and February (N=74). Thirty pregnant females, from all three age categories, were found during this time and they provided 66 foetuses. Both data sets, foetal number and foetal length, were normally distributed allowing for the use of parametric statistical analysis. In examples where variance was not equal among sub-groups, non-parametric statistical tests were used.

5.2.5.2 Pregnancy rate in the study population

Pregnant badgers were obtained between the 2nd January and the 19th February, which was the sampling period for January and February. No pregnant female was found outside this time period. However, sampling in December and March was limited to the 2nd-22nd December and the 1st -16th March respectively; therefore pregnant females may have been missed due to constraints on sampling.

For analysis the period when pregnant females were observed was divided into seven one-week sub-samples. The proportion of pregnant and post partum females, as a percentage of the female study population, was calculated for each week. From weeks 1 to 5 the proportion of pregnant females varied between 60% and 75%, with the highest proportion occurring in week 1 at the beginning of January (Figure 5-30). Following week 5 there was a considerable reduction in the proportion of pregnant females, which was presumably associated with the beginning of parturition as there was a rapid and proportionate increase in post partum females.

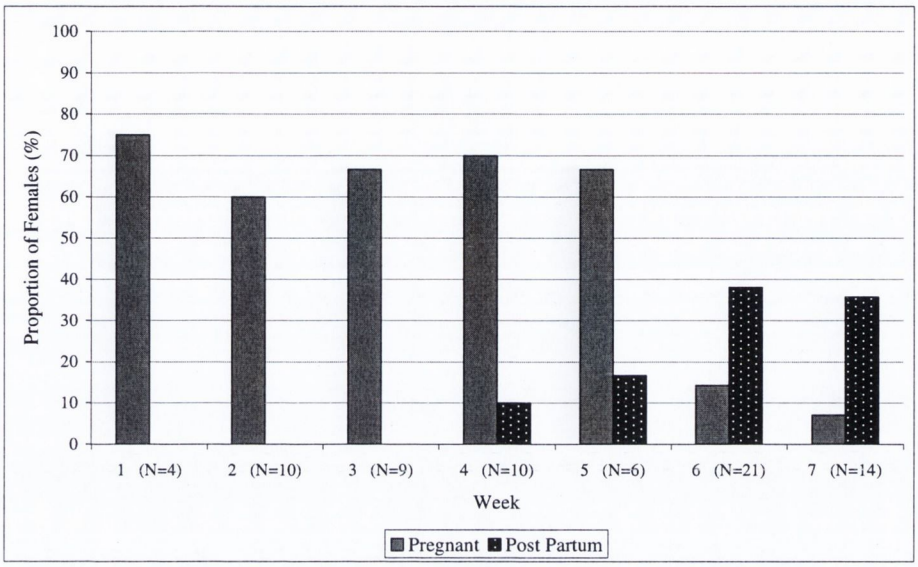

Figure 5-30: Proportion of females, as a percentage of the study population, determined to be pregnant or post partum.

5.2.5.3 Number of Foetuses and implantation dates

The mean number of foetuses was 2.47 ± 0.14 and was similar to the overall median blastocyst number (median = 2).

Using a gestational period of 52 days (Page *et al.*, 1994), and an algorithm incorporating foetal weight as a predictor of gestational age (see Section 2.2.1), and taking into account the date when the female was euthanased, implantation dates were calculated to have ranged from the 19th December to the 14th January (Figure 5-31); that is all implantations occurred within a 26 day period. Parturitions would have begun on the 9th February and the last cubs would have been born on the 7th March.

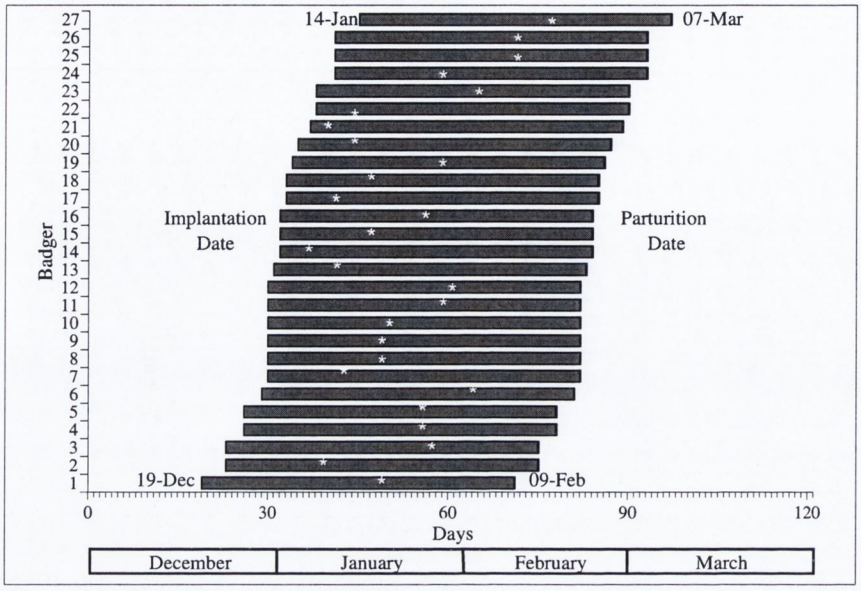

Figure 5-31: Distribution of projected gestation periods for each badger found pregnant, displaying theoretical implantation and projected parturition date. The x-axis displays days - 1st December to 31st March, and ❄ represents actual capture dates.

5.2.5.4 Effect of age

Although the mean number of foetuses was highest for adults and lowest for aged females (Figure 5-32), the differences between the age categories were not statistically significant (ANOVA: F=1.799; d.f.=2; p=0.185).

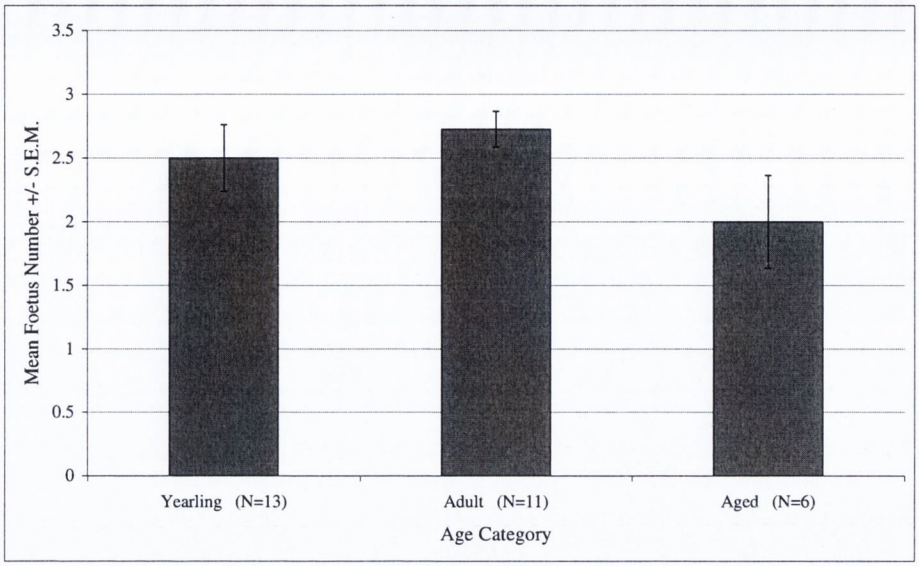

Figure 5-32: Mean foetus number for pregnant females categorised by maternal age. Error bars represent standard error of mean.

5.2.5.5 Importance of body condition during the period of pregnancy

The two measures of body condition, body weight and subcutaneous fat depth, during January and February were greatest in pregnant females (Figure 5-33). There was little variation in body weight and subcutaneous fat depth between non-pregnant females and post partum females. Body weight and subcutaneous fat depth varied significantly with pregnancy status (ANOVA, body weight: $F=12.736$; d.f.=2; $p<0.001$, Kruskall Wallis, SFD: $\chi^2=17.841$; d.f.=2; $p<0.001$). Pregnant females had significantly greater body weights than non-pregnant and post partum females (Bonferroni post hoc test: $p<0.001$ in both cases) and significantly greater subcutaneous fat depths (Dunn's post hoc test: pregnant vs. not pregnant, $p<0.01$; pregnant vs. post partum, $p<0.001$).

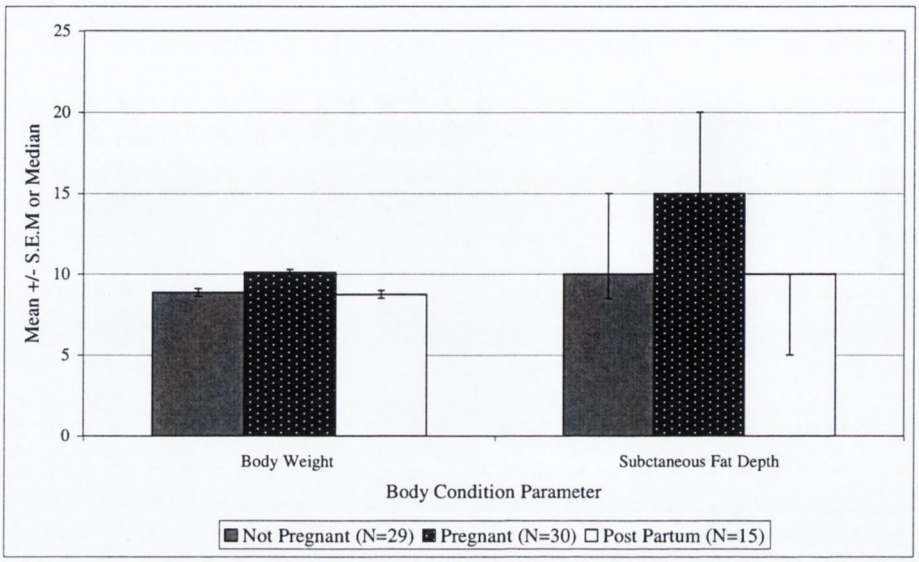

Figure 5-33: Mean body weight and median subcutaneous fat depth of females who were not pregnant, pregnant and post partum. Error bars for body weight represent standard error of mean and inter-quartile range for subcutaneous fat depth.

5.2.5.6 Relationship of foetal number and length to corpora lutea

Although there was a slight positive association between foetus number and corpus luteum number (Figure 5-34), the correlation was not significant (Spearman rank-order correlation: r_s=0.094; N=28; p=0.633).

As the number of foetuses present was not always equal to the number of corpora lutea, a comparison was made between mean foetal length (as a measure of gestational age) and mean corpus luteum surface area for each pregnant female. As foetal length increased corpus luteum surface area decreased (Figure 5-35); and there was a significant negative correlation (Pearson correlation: r=–0.418; N=26; p=0.034). The R^2 value (R^2=0.1748) suggests that nearly a fifth of the variation in foetal length was negatively associated with changes in corpus luteum surface area, with one factor decreasing as the other increased. Despite removal of the outlier (0.81, 114.81; for criteria see Section 2.5) a trend was still apparent (R^2=0.1179), although the correlation was marginally non-significant (Pearson correlation: r=–0.343; N=25; p=0.093).

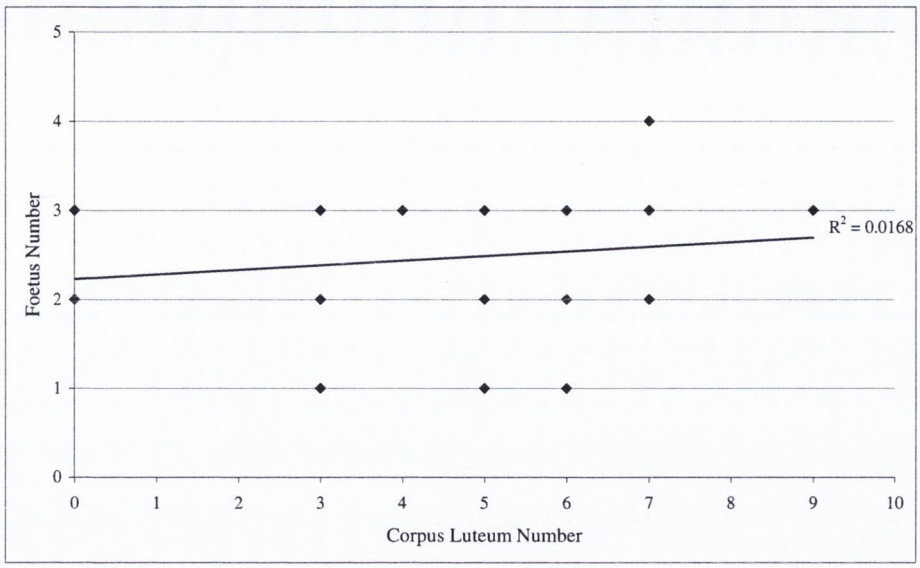

Figure 5-34: Distribution of foetus numbers in relation to corpus luteum numbers for each badger

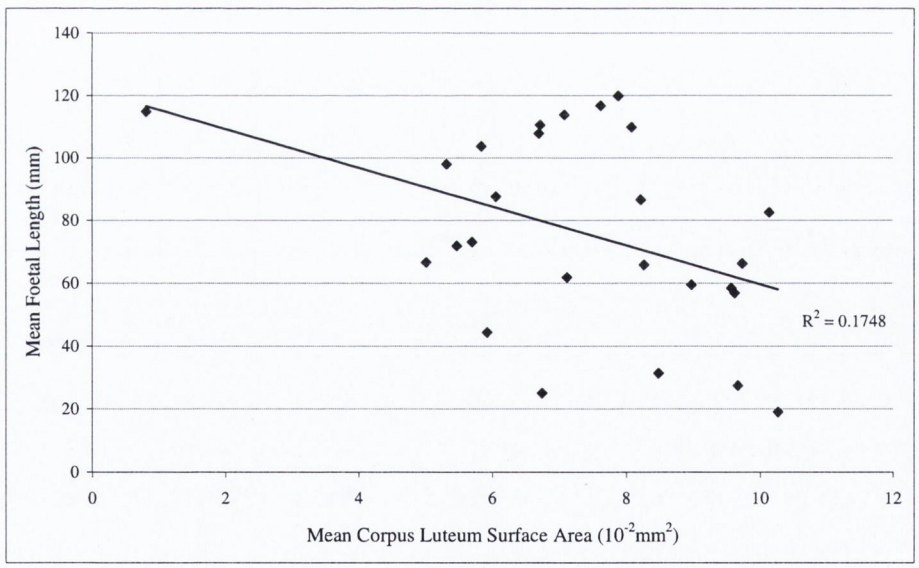

Figure 5-35: Distribution of mean foetal lengths related to mean corpus luteum surface area for each badger.

5.2.5.7 Relationship of foetal number and length to levels of circulating progesterone

Foetal length increased as the level of circulating progesterone decreased (Figure 5-37); with a significant negative correlation (Spearman rank-order correlation: r_s=–0.363; N=48; p=0.011) and a reasonable R^2 value (R^2 = 0.1682). Nearly a fifth of the variation in foetal length was related to changes in circulating progesterone levels. There was no correlation between the number of foetuses and the level of circulating progesterone (Spearman rank-order correlation: r_s=–0.126; N=21; p=0.585) (Figure 5-36).

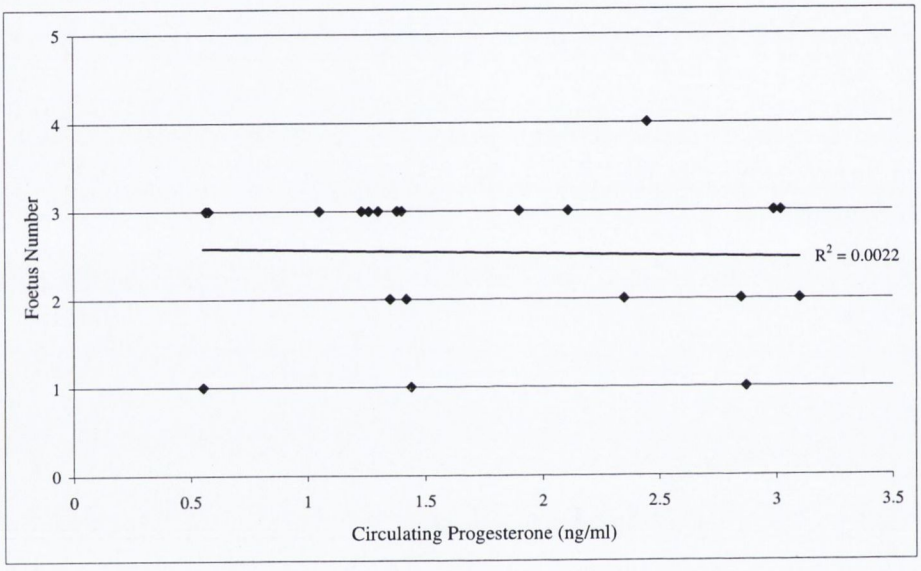

Figure 5-36: Distribution of foetus numbers in relation to circulating progesterone levels for each badger.

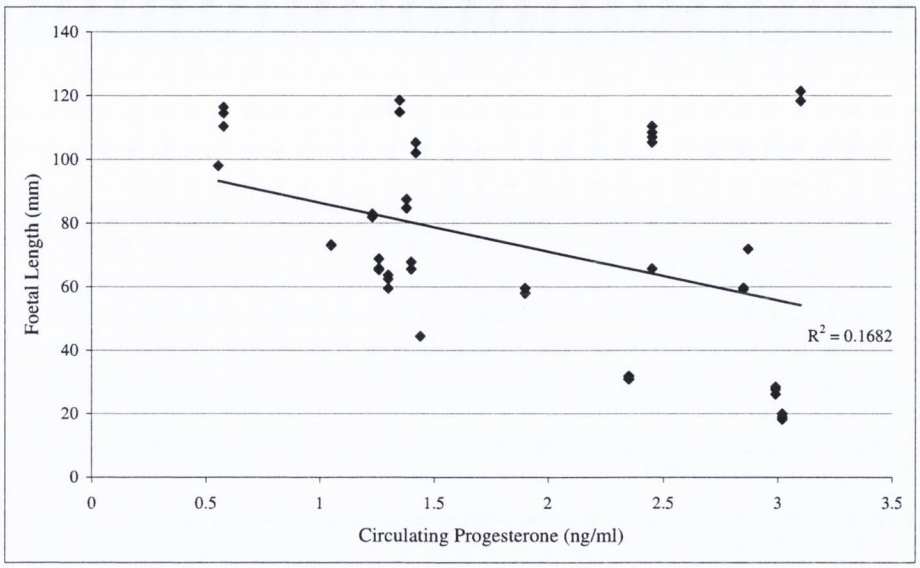

Figure 5-37: Distribution of foetal lengths in relation to circulating progesterone levels for each badger.

5.2.6 Lactation

5.2.6.1 Description

Females were assigned to one of four categories or stages of lactation. That is, females were either "lactating" (thick secretory area, secretory units expanded containing extensive secretions), showed "mammary development" that was indicative of either pre-lactation or post-lactation involution (thick secretory area, secretory units not expanded and containing no secretions), were mature with no mammary development, "not lactating" (secretory area reduced, but with lobules that are evidence of previous pregnancies) or were "under-developed" (no secretory area and lobules absent).

5.2.6.2 Lactation rate of study population

Evidence of active lactation in females was observed during a seven-month period, from January to July (lactating, Figure 5-38(a)). The proportion of females lactating was low in January and increased to a peak in June (62%) and remained high in July. There was a sudden and dramatic reduction to zero in August. No lactating females were observed during the remainder of the year.

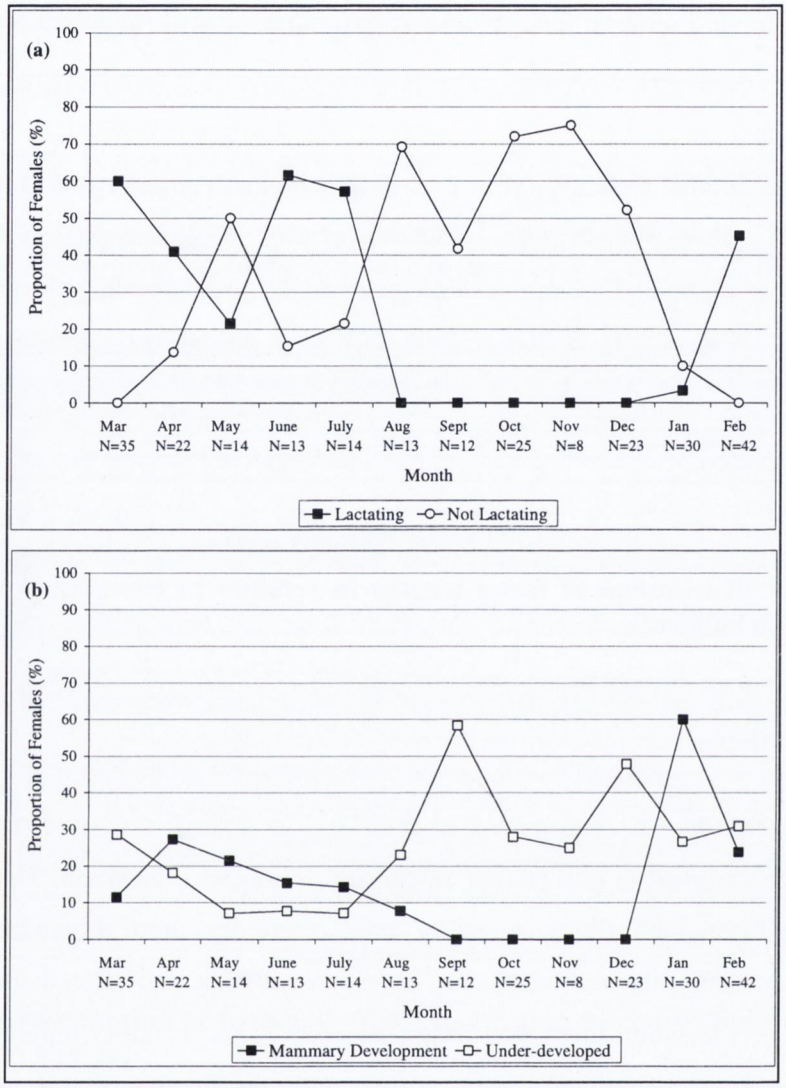

Figure 5-38: Proportion of females, as a percentage of the study population, displaying evidence of (a) lactating and not lactating and (b) mammary development and under-development of mammary tissue

Females with mammary development were present in the eight months from January to August (mammary development, Figure 5-38(b)). The highest proportions occurred in January, (pre-lactation) and gradually decreased until August (post lactation).

Females with no development were present from May to December (not lactating, Figure 5-38(a)), and those with pre-pubescent mammary glands were present throughout the year

(under-developed, Figure 5-38(b)). During September to December all females belonged to either one of these two categories only.

5.2.7 Effect of group size on reproductive behaviour

5.2.7.1 Description

As described in Chapter 3 individuals were assigned to a social group based on the sett where they were trapped. Groups of >1 individual were either single sex or mixed sex groups, and included groups with more than one sexually mature female. It has been proposed that where there are more than one mature female within a social group, a reproductive hierarchy may develop; with reproductive suppression of subordinate members leading to reduced reproductive success (Cresswell *et al.*, 1992, Kruuk, 1989, Woodroffe & MacDonald, 1995b). For the analysis of the effect of group size, females (N=194) were classified according to the number of co-habiting females within their social group. Females belonged to groups with two, three or four females. Incidentally there were a high proportion of solitary females. All females were classified as either reproductively successful (blastocysts or foetuses present) or unsuccessful (blastocysts and foetuses absent).

5.2.7.2 Reproductive activity and group size

A comparison was made between the proportion of reproductively successful females where the group contained no other mature female and those with ≥1 (Figure 5-39). There was a decrease in the proportion of successful females as the number of females in the group increased. Groups with 4 females had the lowest success rate (25%) while the greatest success rate was in groups with 2 mature females (42%) and those in single female groups (39.72%).

In an analysis of groups with two female members, where only adult and aged badgers were included (N=12); there was only one instance where both females were successful (8.33%), seven cases where only one female was successful (58.33%), and four cases

were neither of the females were successful (33.33%). There was only one group with three female members and none were found to be pregnant.

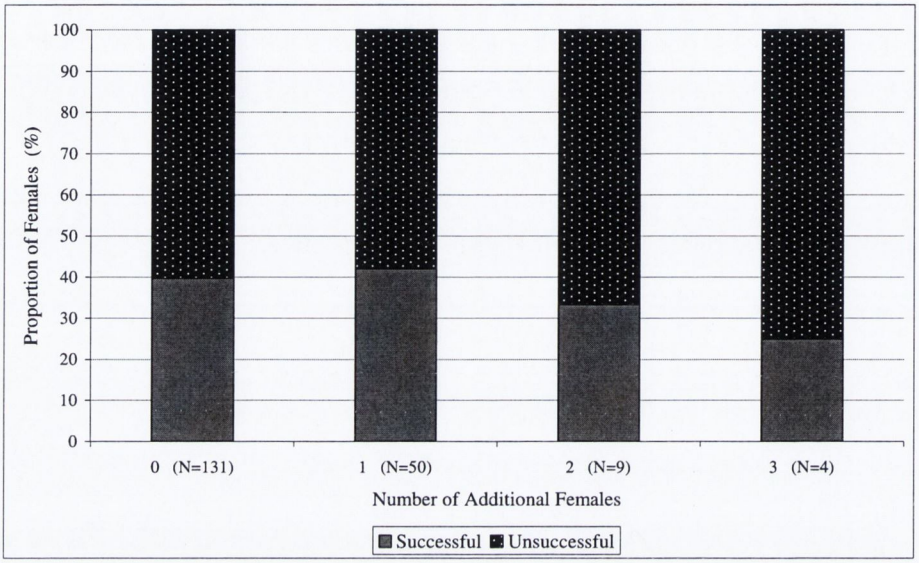

Figure 5-39: Variation in the proportion of reproductively successful females in relation to the number of co-habiting females within the social group

5.2.8 Relationship between past and present reproductive performances

5.2.8.1 Description

The numbers of placental scars on each uterine horn were recorded and used to determine past reproductive success. It was not possible to detect placental scars in pregnant females, therefore analysis excluded data from January and February. Placental scars were categorised as recent or faded (see Section 2.2.1); females could have both categories of placental scar present. Females were categorised as having recent placental scars (N=71), faded placental scars (N=7) or both recent and faded placental scars (N=20). There was no significant difference between left and right uterine horns in the number of placental scars (t-test: t=–0.1.143; d.f.=340; p=0.254). The data set was normally distributed allowing for the use of parametric statistical analysis. In examples where variance was not equal among sub-groups, non-parametric statistical tests were used.

5.2.8.2 Proportion of females with blastocysts and previous reproductive success

Females with no placental scars were considered either to have never bred before, that is first time breeders (primiparous), or to have not actively bred in at least the past 2-3 seasons. Blastocysts were present in 32.88% of the seventy-three individuals in this condition (Figure 5-40); of these individuals 73% were yearlings and consequently primiparous. The presence of recent placental scars was taken as evidence that females had successfully completed a pregnancy in the previous year. Of these individuals, 49.30% carried blastocysts; that is, they were reproductively active in two consecutive years.

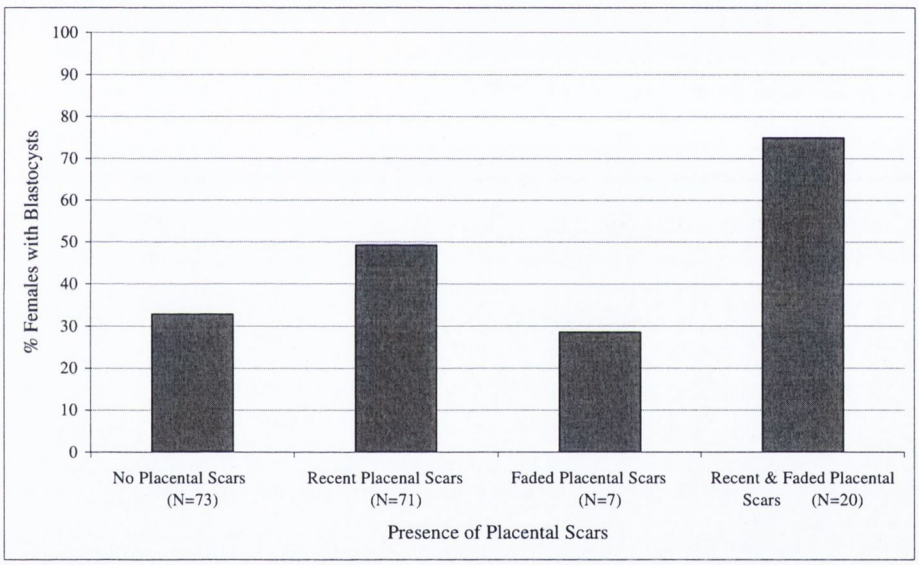

Figure 5-40: Proportion of females carrying blastocysts in relation to previous reproductive history as assessed from the presence of placental scars.

Of the seven females with faded placental scars only 28.57% were reproductively active in the current year (Figure 5-40). There were twenty individuals who had both recent and faded placental scars; evidence that they had been pregnant at least twice. Of these individuals, 75% were carrying blastocysts in the current year; evidence that they had been reproductively successful over three consecutive years.

5.2.8.3 Placental scar numbers compared to present blastocyst numbers

It was possible to compare the number of foetuses successfully carried to term in previous pregnancies using recent placental scars, and the number of potential foetuses in the current breeding season from the number of blastocysts present. This was used to determine the variation in fecundity between successive reproductive seasons. The median number of recent placental scars was equal to the median number of blastocyst (Figure 5-41). When placental scar numbers were compared to blastocyst numbers for individuals there was no significant difference (Wilcoxin signed ranks: Z=0.156; N=15; p=0.876). Therefore, fecundity rates do not appear to vary between seasons.

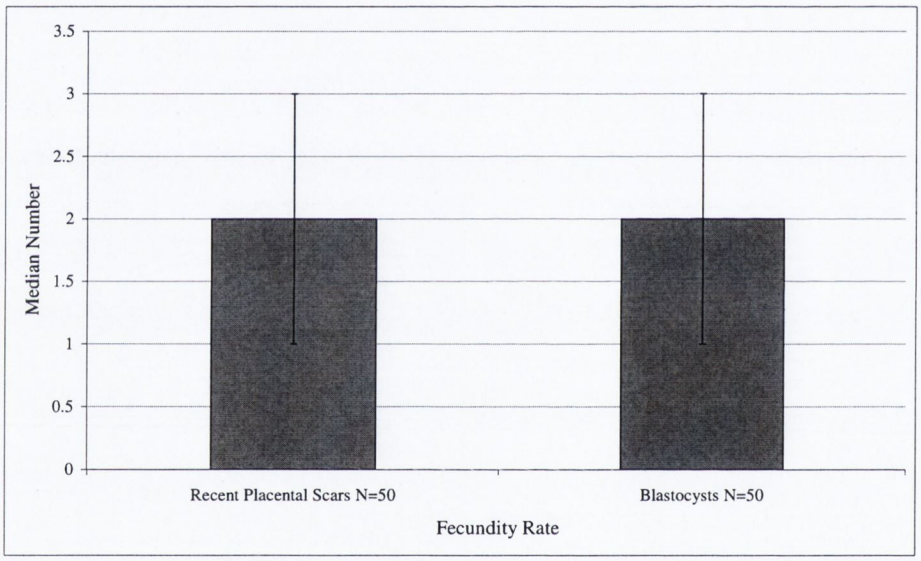

Figure 5-41: Comparison of median fecundity rates for previous pregnancies (recent placental scars) and current pregnancies (blastocysts). Error bars represent inter-quartile range.

5.2.9 Reproductive Potential

5.2.9.1 Description

By describing the proportion of females displaying different reproductive states, it was possible to estimate the level of reproductive success and to postulate the reproductive potential of the badger population being studied. Four different (and non-exclusive)

states were identified in the reproductive cycle: presence of corpora lutea, presence of blastocysts, presence of foetuses, and lactation. The presence of corpora lutea indicated that ovulation had occurred and was observed all year round. The presence of blastocysts indicated that mating and fertilisations had occurred, and blastocysts were present from March and December. Pregnancy, that is the presence of foetuses, occurred during January and February. Finally, lactation occurred during the seven months from January to July.

5.2.9.2 Estimate of reproductive performance of the sample population

There was a progressive reduction in the proportion of females in each reproductive state as the population progressed through the reproductive cycle. Adult females had the highest proportion for corpora lutea and blastocysts present, yearlings had the highest proportion for foetuses and aged badgers had the highest proportion for lactation (Figure 5-42). Ovulation (corpora lutea present) was observed to have occurred in 79.07% of females, 44.19% of females were successfully fertilised (blastocysts present), 40.54% of females successfully implanted blastocysts and became pregnant and a similar proportion

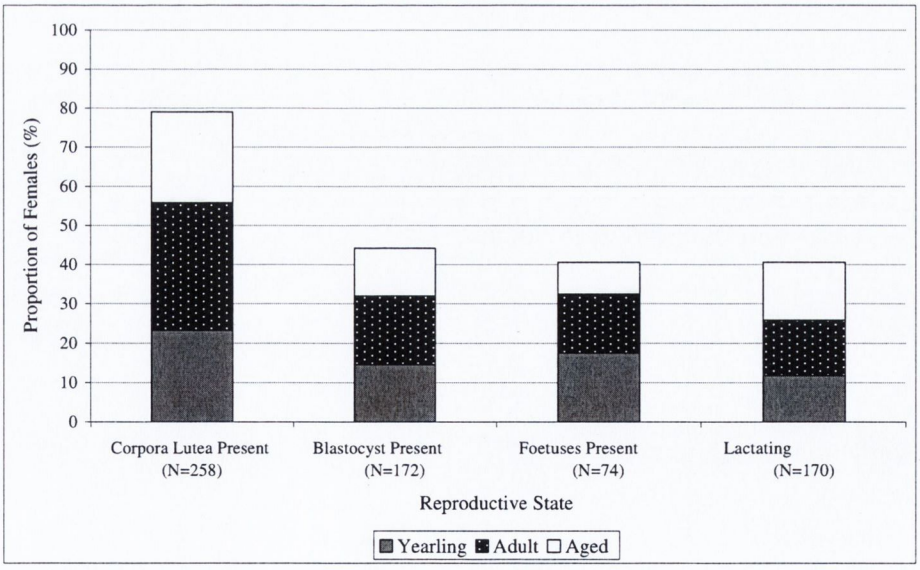

Figure 5-42: Reproductive performance of the three age categories, as a percentage of the sample population

actively lactated, 40.59%. That the proportion of pregnant and actively lactating females was similar would suggest a high proportion of foetuses survived to term and that a similar proportion of pregnancies resulted in live births and survival of (some) cubs.

The low proportion of females successfully breeding (i.e. resulting in the presence of live cubs) appears to be due to a failure of fertilisation, namely, ovulation with no establishment of blastocysts. The proportions in the latter three stages remained fairly comparable; suggesting that once fertilisation occurs, most females complete the breeding cycle successfully. Thus, approximately 40% of mature females bred successfully and as the number of blastocysts per female was similar to the number of foetuses, the potential was maintained through to parturition.

5.3 Summary

- Based on evidence from histological examination of the endometrium and vaginal epithelium, and from pre-ovulatory follicle number oestrual females were most common in February, March and November. April June and August had high proportions of oestrual females but relatively low numbers of pre-ovulatory follicles. However, it is still possible that these are months in which females come into oestrus.

- Ovulation frequencies were higher during the first half of the breeding year, March to August (~90%), than the second half, September to February (~80%).

- Mean corpus luteum number increased consistently during the year suggesting continual ovulation. Conversely, corpus luteum surface area declined from March to November, but increased to its greatest areas in January.

- Corpus luteum number and surface area did not vary with successful fertilisation. Both females with bastocysts and those without had similar corpora lutea number and dimensions during the same period of March to December. During January and

February pregnant females and those found not to be carrying foetuses had similar corpus luteum number but corpus luteum dimensions were greater for pregnant females. Post partum females during the same period (January-February) had significantly fewer and smaller corpora lutea than pregnant females; with the number and dimensions being indicative of females from the beginning of the breeding year.

- Blastocysts were detected between March and December.

- Fertilisation frequency gradually increased from March to its greatest proportions in August. Thereafter, proportions remained reasonably constant (60-70%).

- Blastocyst number remained relatively constant from March to July, followed by an increase to its maximal value in October-November. There was a decline in blastcyst number in December; prior to the period of implantation. Blastocyst diameter gradually increased from March to December.

- Between June and December there were blastocysts with small diameters, indicative of earlier months, which could be interpreted as a sign of continual ovulation and fertilisation into the latter part of the year.

- 18% of females with blastocysts showed histological evidence of being in oestrus.

- 68% of females possessing blastocysts had excess corpora lutea.

- Pregnant females were identified exclusively between the 2nd January and the 19th February. The highest proportion of pregnant females per month occurred during the first week of January (75%). There was a rapid increase in the number of post partum females at the beginning of February.

- Mean foetus number was 2.47 ± 0.14, which was comparable to the overall median blastocyst number (median = 2)

- Implantations all occurred within a 26 day period, from the 19th December to the 14th January. Based on foetal length, parturitions would have begun on the 9th February with the last cubs being born on the 7th March.

- Pregnant females had significantly higher body weights and subcutaneous fat depths compared to non-pregnant and post partum females.

- Lactating females were identified during a seven month period from January to July. Proportions were low in January (3.33%) and high in March, June and July (51%-62%). Between August and December the proportion of lactating females dramatically decreased to zero.

- Yearlings had the lowest frequencies of ovulation, fertilisation, gestation and lactation. However, those that were successful had comparable numbers or corpora lutea, blastocysts and foetuses.

- Circulating progesterone levels were higher for females with corpora lutea than those without. Metoestrus/dioestrus begins with the formation of the corpus luteum and this period of the oestrous cycle was associated with the highest levels of circulating progesterone. Progesterone levels and ovarian weight were also related to corpus luteum surface area. Conversely, blastocyst diameter and foetal length (representing foetal age) were negatively associated with corpus luteum surface area. Foetal length was also negatively associated with circulating progesterone levels. This suggests that as delayed implantation and pregnancy advanced, corpus luteum surface area and progesterone, in the case of foetal development, declined.

- There was a decline in the proportion of pregnant females as the number of mature females in a social group increased. Of the few examples of groups with two mature adult females (N=12), 58.33% had only one pregnant female within the group.

- 75% of females with recent and faded placental scars were carrying blastocysts during the study year. Assuming that recent and faded placental scars resulted from separate

gestations, this suggests that females become pregnant over three breeding season. It is not possible to assess if these gestations were successful.

- Fecundity did not appear to vary between seasons.

- Failure to fertilise, that is, ovulation with no establishment of blastocysts, appears to be responsible for the largest reduction in breeding success.

- Following fertilisation most females complete the breeding cycle successfully. Approximately 40% of the sexually mature females within the study population bred successfully.

5.4 Discussion

Female badgers can be considered to be polyoestrous. This reproductive strategy has a number of implications: continued opportunities to conceive; opportunities for superfetation and polyandry to occur; and production of additional corpora lutea which may provide an added source of progesterone.

Badgers have been described as a monoestrous species, with their breeding season confined to February and March (Canivenc & Bonnin, 1981). More recent studies, however, including strong support in the present study, show that badgers are polyoestrous, with oestrous cycles occurring throughout the breeding year until implantation and conception in December (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Page *et al.*, 1994). In the present study, ovulations occurred in February, March and November, based on the relatively high number of pre-ovulatory follicles, proliferation of the endometrium and vaginal cornification. April, June and August were also reported to be months in which females ovulate based solely on the condition of the endometrium and the vaginal epithelium. No definite estimates of the frequency or timing of oestrous cycles in the badger could be made from the data available. However, for the

purpose of this discussion, the oestrous cycles will be divided into three time periods. The first oestrous cycles were considered to occur early in the year during late winter-spring (February, March and April), additional oestrous cycles occur in summer (June and August), and the final, additional oestrous cycles occurs in late autumn (November).

In the present study the first oestrous cycles occurred early in the year, between February and April. This was evidenced by the high numbers of pre-ovulatory follicles, the histological condition of the uterine endometrium and vaginal epithelium, the increase in ovulation rate (the proportion of females with corpora lutea) and the increase in fertilisation rate (the proportion of females with blastocysts). These observations are consistent with previous observations in other parts of Europe (between January and April (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Neal & Harrison, 1958, Page *et al.*, 1994, Paget & Middleton, 1974, Whelan & Hayden, 1993). A proportion of the oestrual females during February and April would have been in a state of post partum. The advantages of breeding during such a costly period may be considerable: acceptance by resident males (Ahlund, 1980), high levels of male fertility (see Chapter 6), increased group cohesion (Ahlund, 1980) and increased male competition leading to effective female choice (Sandell, 1990).

Oestrous cycles continue throughout the breeding year, significantly contributing to the fertilisation rate of the population (Cresswell *et al.*, 1992, Mondain-Monval *et al.*, 1980, Neal & Harrison, 1958, Page *et al.*, 1994). For the present study these additional oestrous cycles occurred during the summer (June and August) and late in the autumn (November). Evidence of additional ovulations included: histological changes in the uterus and vagina; high ovulation rates coupled with a steady increase in corpora lutea number; and an increase in fertilisation rate coupled with a steady increase in blastocyst number. These additional oestrous periods were responsible for providing further opportunities to conceive as was evident from the increase in fertilisation rate to its maximal proportions during this period in the present study (August: 67%), a previous Irish study, (Whelan & Hayden, 1993 - September: 80-90%) and a study from the south-west of England (Page *et al.*, 1994 - August: 94%). Earlier ovulations may be more influential in increasing fertilisation rates to their maximum levels in other populations

(South-west England, Cresswell *et al.*, 1992 - March: ~80%; Sweden, Ahlund, 1980 – June: 90-95%)

In addition to blastocyst numbers increasing during September to December, further evidence that ovulations continue throughout the breeding year was provided by the presence of a wide range of blastocyst sizes, including blastocycts with small diameters. These observations are consistent with the majority of studies in Europe (Ahlund, 1980, Cresswell *et al.*, 1992, Page *et al.*, 1994, Whelan & Hayden, 1993). The presence of small blastocysts was taken to be indicative of recent ovulations and fertilisations. In the present study, females in this condition were assigned to one of three categories: yearlings where the numbers of corpora lutea corresponded to the number of blastocysts, suggestive of first-time breeders (2% of fertilised females during Sept-Dec) (Cresswell *et al.*, 1992), adults that had an excess of corpora lutea owing to either ovulation without fertilisations or loss of blastocysts (5% of fertilised females during Sept-Dec) (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Neal & Harrison, 1958), or adults that had blastocysts of two distinct sizes and that had a corresponding number of corpora lutea compared to the number of blastocysts (7% of fertilised females during Sept-Dec) (Ahlund, 1980, Cresswell *et al.*, 1992). The latter group provide evidence of possible superfetation, that is, conception during pregnancy (Cresswell *et al.*, 1992, Yamaguchi *et al.*, 2006). Further evidence for superfetation was provided by females who had proliferation of the endometrium and vaginal cornifiaction while possessing potentially viable blastocysts (18% of fertilised females during Mar-Dec). Similar observations of cyclic changes to the vaginal epithelium (Neal & Harrison, 1958), folliculogenesis (Harrison & Neal, 1956), and the presence of ova (Harrison & Neal, 1956, Page *et al.*, 1994) in successfully fertilised females have previously been described

Multiple ovulations over an extended breeding season allow for superfetation and increase opportunities for polyandry to occur. Superfetation could be an adaptation leading to increased female fitness and reduced infanticide by resident males through paternity confusion. Superfetation and increased opportunities for polyandry can be considered to be additional advantages of delayed implantation (Yamaguchi *et al.*, 2006,

Yamaguchi *et al.*, 2004). Natural superfetation in the badger has been previously described in Ireland, the south-west of England and Sweden (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). As with the present study, examples of superfetation in Sweden were relatively rare, with only one example being described (Ahlund, 1980). Previous studies in Ireland and the south-west of England described relatively high frequencies at 14% and 21%, respectively (Cresswell *et al.*, 1992, Whelan & Hayden, 1993). The relative inactivity of the corpora lutea during the period of diapause has been reported to be responsible for superfetation in the American Mink (*Mustela vison*) (Venge, 1973 c.f. Yamaguchi, 2004, (Sundqvist *et al.*, 1988). The brief delay in implantation reported for the mink prolongs the window of opportunity for mating and superfetation, and in turn provides greater opportunities for polyandry or mixed paternity litters which leads to an improvement in female fitness (Yamaguchi *et al.*, 2004). Yamaguchi *et al.* (2006) suggested that if superfetation, and hence increased opportunities for polyandry, was regularly exhibited in the badger it may be facilitated by the long period of delay.

There appeared to be two patterns of corpora lutea production: excess corpora lutea in the early part of the year and those produced in the late autumn. Progesterone levels were observed to be relatively high throughout May to December (see Section 4.2.3.2). During the early part of the year there was an accumulation of small corpora lutea, with the numbers usually in excess of the number of blastocysts. The presence of additional corpora lutea was also observed by Page *et al.* (1994) in badgers in south-west England. Progesterone levels must be sustained during the period of diapause for the maintenance of the diapausing blastocyst (Bonnin *et al.*, 1978, Mead, 1993). Although these additional corpora lutea may have resulted from failed fertilisations or loss of blastocysts, they may also have developed after ovulations, in a similar strategy as that adopted by the brush-tailed porcupine (*Atherurus africanus*, Gray 1842), a mono-embryonic species (Jori *et al.*, 2002). This species uses superovulation to produce additional corpora lutea, which are used to produce sufficient progesterone to sustain the long period of gestation (Jori *et al.*, 2002, Mossman & Duke, 1973). In the present study there was evidence of females entering oestrus while possessing blastocysts, which may provide evidence of superfetation. Alternatively, this mechanism may provide support for the production of

additional corpora lutea as a source of progesterone. It is also possible that additional corpora lutea may develop from secondary follicles without the requirement of ovulation. Ovulations in late autumn (November) may provide additional blastocysts but may also have an alternative or additional function of providing females with additional corpora lutea. As with a previous study in south-west England (Page *et al.*, 1994), neither ovulation rate nor fertilisation rate increased during autumn, despite an increase in both corpora lutea and blastocyst numbers. These corpora lutea may supplement or re-establish progesterone levels necessary for implantation to occur, a theory supported by Harrison & Neal (1956).

Mean corpora lutea number continued to increase during the winter months (December-February). It is unlikely that these resulted from ovulations as all implantation occurred from mid-December to mid-January with gestation continuing until early February/March. Therefore, these may also be additional corpora lutea derived from secondary follicles. Besides the presence of remnants of the retained oocyte, accessory corpora lutea are morphologically identical to true corpora lutea, making them hard to differentiate (Jori *et al.*, 2002). Interestingly, accessory corpora lutea have been reported in the mink through the partial transformation of the granulosa cells of non-ovulated corpora lutea (Mossman & Duke, 1973).

The females that successfully implanted had similar numbers of corpora lutea to those that failed, but the corpora lutea were larger. This increase in size was also reported by Page *et al.* (1994). Full luteal differentiation involves doubling of the luteal cells and is accompanied by increased progesterone secretion. Luteal differentiation is necessary for implantation and renewed embryonic development (Mead, 1981). Large corpora lutea were indicative of luteal differentiation, a process which did not occur in the females that failed to implant. Therefore, the lack of luteal differentiation would have resulted in blastocysts not implanting and being lost. Alternatively, blastocysts may have been lost earlier in the breeding season and the corpora lutea which remained were in a state of retrogression.

In most species, progesterone, either secreted from corpora lutea or from uterine sources, is necessary for the maintenance of pregnancy, with the establishment of the placenta coinciding with a peak in progesterone levels (Canivenc & Bonnin, 1981). In the badger, the placenta undergoes rapid development between days 15-30 of the gestation period and is thought to be involved in progesterone secretion (Mead, 1993). In the present study, implantation occurred between mid-December and mid-January, with a surge in progesterone secretion in January (see Section 4.2.3.2), possibly associated with placental development. However, in the badgers examined in this study, the latter stages of pregnancy were associated with a decrease in both the size of the corpora lutea and the levels of circulating progesterone (see Section 4.2.3.2). This may indicate that, in the badger, either progesterone is not necessary for the maintenance of pregnancy in the later stages of gestation, or that placental progesterone is more influential than luteal progesterone during this time. Harrison and Neal (1956) reported that although corpora lutea persist until the end of gestation, they show signs of involution mid-pregnancy.

Unlike the extended period of the breeding season, the period of implantations and gestation was very truncated. Based on foetal weights, implantations were estimated to have all occurred within a 26-day period from mid-December to mid-January with parturitions occurring during February and March (a gestation period of 52-days). Previous studies have described cubs being born during the first three months of the year with the majority of parturitions occurring in late January and February (Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). Lactation in reproductively successful females ceases in June/July in both the present study and previous studies (Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). This is consistent with the predicted parturition dates and the time when cubs are believed to become fully independent (at 12 weeks of age) (Neal & Cheeseman, 1996). Therefore, it would be recommended, on welfare grounds, that DAFF should operate a closed season on badger removals for a determined period between January and June. The reproduction rate was lower than was expected. Mean foetal number was 2.5 for the study population, lower than that described in a previous Irish study (Whelan & Hayden, 1993, mean =2.9) and that described for the south-west of England (Neal & Harrison, 1958, mean =3.1; Page *et al.*, 1994, mean=2.9).

Reproductive failure, that is, the failure to produce live offspring, occurred at all stages of the female reproductive cycle. Failure of fertilisation, that is, ovulation without the establishment of blastocysts or loss of blastocysts during the period of diapause, was responsible for the greatest proportion of the losses. This was supported by: higher ovulation rates than fertilisation rates throughout the year and the presence of greater numbers of corpora lutea than blastocysts. Such discrepancies between ovulation and fertilisation were consistent with previous studies in the south-west of England and in Sweden (Ahlund, 1980, Cresswell *et al.*, 1992, Neal & Harrison, 1958, Page *et al.*, 1994). Females with additional corpora lutea represented those individuals that: have no blastocysts owing to infertile matings (Ahlund, 1980, Cresswell *et al.*, 1992, Harrison & Neal, 1956, Neal & Harrison, 1958), had produced more ova than were fertilised (perhaps during >1 oestrous cycle) (Ahlund, 1980, Cresswell *et al.*, 1992), and individuals that have lost either all or a proportion of their blastocysts following fertilisation (Cresswell *et al.*, 1992, Harrison & Neal, 1956). Contrary to a previous study in Sweden (Ahlund, 1980) corpus luteum numer was not greater in females possessing blastocysts compared to those without. This suggests that in the present study the number of ova liberated had no effect on fertilisation rate.

Gestation and lactation rates for the study population were similar to the fertilisation rate. That is, in contrast to previous studies, reproductive failure was not due to loss of blastocysts or losses during pregnancy. A previous study in Ireland found foetal/cub mortality was the greatest influence on reduced reproductive performance. In that study it was reported that implantation frequencies were almost twice lactation frequencies (Whelan & Hayden, 1993). In the south-west of England, despite similar levels of fertilisation, the greatest losses occurred during implantation (Cresswell *et al.*, 1992, Page *et al.*, 1994). One of the studies in south-west of England, reported that mean blastocyst number decreased prior to implantation (Cresswell *et al.*, 1992), this also occurred in the present study. It was females with the greatest numbers of blastocysts that were most likely to successfully implant, as mean foetal numbers were slightly higher than blastocyst numbers.

In the present study it appeared that once blastocysts were maintained the majority of females went on to complete the breeding cycle, with approximately 40% of sexually mature females becoming pregnant. This is comparable with frequencies described for a previous Irish study (Whelan & Hayden, 1993: 35-40%), but greater than that described in the south-west of England (Cresswell *et al.*, 1992: 30%). However, these reproductive rates were much lower than those recorded for low density populations in Sweden, where 90-95% of the adult female population implanted successfully (Ahlund, 1980) and Donaña area of Spain where 65% if females bred successfully (Revilla *et al.*, 1999). Post-natal losses were not calculated for any of the reproductive studies mentioned above so it was only possible to estimate reproductive success from the rate of pregnancies. However, based on behavioural observations of a population in the south-west of England it is believed that pre-weaning cub mortality is low (Woodroffe & MacDonald, 1995b).

The published literature strongly supports the hypothesis of a density dependent constraint on reproduction. In high density populations, close to the carrying capacity of the habitat, a reduction in fecundity was seen as group size increased (da Silva *et al.*, 1993, MacDonald *et al.*, 2002a, Tuyttens *et al.*, 2000b). Studies of high-density populations in the south-west of England showed that, regardless of group size, the majority of groups had only one litter of cubs (Cheeseman and Harris unpublished c.f. Cresswell *et al.*, 1992) In the present study, although not strongly supported by the data, there was some indication that only a single female within a group may reproduce successfully. Moreover, the proportion of pregnant females within social groups declined as the number of mature females in the group increased. The reproductive performance of the study population is similar to that of the low density populations in Scotland and south-western Spain. In these populations it was also observed that in groups with multiple mature females only one female bred successfully. However, it was not clear whether this was due to reproductive suppression or low food abundance, or both (Kruuk, 1978, Revilla *et al.*, 1999).

Age had less of an effect on breeding success than described previously. Although yearlings had the lowest frequencies of ovulation, fertilisation, gestation, and lactation, those that were successful had comparable numbers of corpora lutea, blastocysts and

foetuses. In previous studies, fertilisation, including the number of ova successfully fertilised, implantation rates and gestation rates were lower for younger females (Ahlund, 1980, Page *et al.*, 1994, Whelan & Hayden, 1993). In the present study nearly half of the pregnant females were yearlings. However, lower lactation rates may indicate that yearlings may experience higher pre- and post-natal losses.

6 MALE REPRODUCTIVE TRACT MORPHOLOGY & SEASONALITY

6.1 Introduction

Testicular dimensions are often used as an indicator of the potential for sexual activity; with increased mass indicative of a sexually active male and reduced mass an inactive male (Ahlund, 1980, Page *et al.*, 1994). This chapter will focus on the morphological changes of the male reproductive tract, namely changes in the testes and the seminal vesicles, during the breeding year and with animal age.

High concentrations of testosterone are necessary for spermatogenesis to occur; however high concentrations are not required continuously so testosterone is released in short bursts every few hours. The level of circulating testosterone, and consequently spermatogenesis, is regulated by a negative feedback loop via the hypothalamus (Senger, 1997). The cycle of circulating testosterone will be examined and related to demographic factors (time of year and animal age) and physiological factors (changes in testicular weight, and levels of spermatogenesis).

Spermatogenesis takes place in the seminiferous tubules of the testes and involves transformation of the primary germ cells, spermatogonia, to highly specialised spermatozoa. Spermatogonia undergo a number of mitotic divisions resulting in the formation of primary spermatocytes. Primary spermatocytes become haploid spherical spermatids following two meiotic divisions. The last stage in development of the

spermatozoa is the production of a flagellum and mitochondrial helix for motility (Bacha & Bacha, 2000, Dellmann & Eurell, 1998, Senger, 1997).

After formation the spermatids are released into the lumen of the seminiferous tubules. They are transported to the epididymides via the ductuli efferentes. Final maturation of the spermatozoa occurs in the proximal parts of the epididymides, the caput and cauda epididymis; the mature functional spermatozoa are then stored in the cauda epididymis. Cohorts of spermatogonia are continuously undergoing development. Within the seminiferous tubules, spermatogonia are located at the periphery. As they transform into spermatozoa they migrate towards the lumen. It is possible to observe these developmental stages histologically (Bacha & Bacha, 2000, Dellmann & Eurell, 1998, Senger, 1997). In this chapter I will show that, to some extent, the male badger is a seasonal breeder by the presence or absence of spermatozoa in the seminferous tubules of the testes and the ducts of the epididymides at different times of the year. The number of spermatozoa present in the testes and epididymides were allocated an "abundance score". Changes to abundance score were examined with reference to demographic factors (time of year and age) and physiological factors (change in testicular weight and levels of circulating testosterone).

6.2 Results

6.2.1 Testicular Weight and Diameter

6.2.1.1 Description

Weight and diameter of the left and right testes were recorded for each individual. The four data sets were normally distributed, allowing for the use of parametric statistical analysis. In examples where variance was not equal among sub-groups, non-parametric statistical tests were used.

There was no significant difference between left and right testicular weights, nor testicular diameters (t-test: testicular weight, t=0.646; d.f.=580; p=0.518; testicular diameter, t=–0.004; d.f.=560; p=0.996). Therefore, mean testicular weight and mean testicular diameter were used to represent each individual. As the left and right testes did not differ significantly in weight or diameter, in those cases where only one testicular weight or diameter was available, that is, where testes were lost or damaged during the post mortem procedure, the single value was used in the analysis. This provided a sample size of N=291 for mean testicular weight and N=288 for mean testicular diameter.

6.2.1.2 Effect of time of year

There was little animal-to-animal variation in the mean monthly values of the two parameters. Testicular weights and diameters followed similar trends through the months with testicular weight showing greater variation across the breeding year. Both testicular weight and diameter decreased gradually from March to November; followed by quite a sharp increase during the winter to a peak in February for testicular weight and April for testicular diameter (Figure 6-1). There was a significant effect of month on mean testicular weight and diameter (Kruskall Wallis: testicular weight, χ^2=113.11; d.f.=11; p<0.001; testicular diameter, χ^2=79.41; d.f.=11; p<0.001).

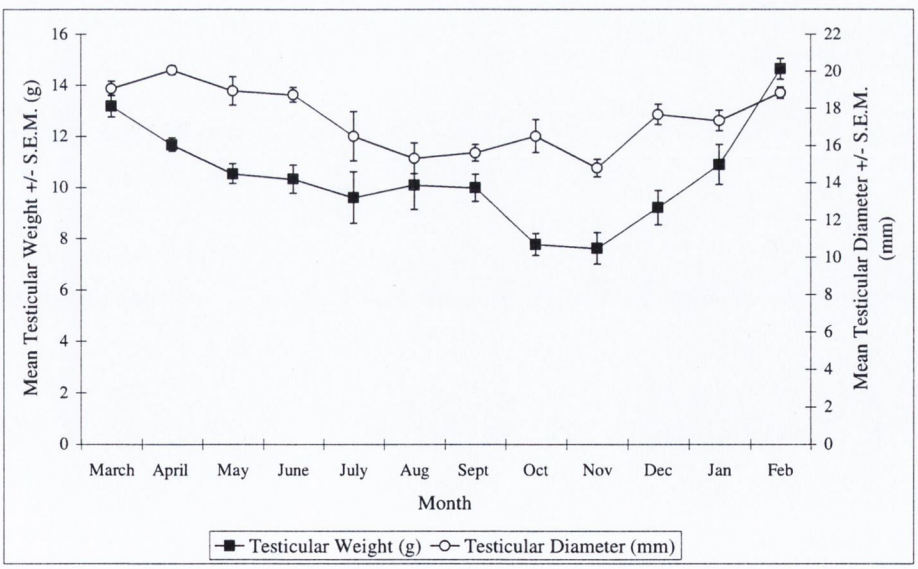

Figure 6-1: Variations in mean testicular weight and diameter by month. Error bars represent standard error of mean

The peak in testicular weight occurred in February (Figure 6-1), and was significantly greater than the nine months, May to January (Dunn's post hoc test: Feb vs. June & Aug, $p<0.01$; $p<0.001$ in all other cases). January, March and April were all months associated with relatively high testicular weights and were significantly greater than some of the lowest monthly testicular weights (Dunn's post hoc test: Jan vs. Nov, $p<0.05$; Mar vs. Sept, $p<0.05$; Mar vs. Dec, $p<0.01$; Mar vs. Oct & Nov, $p<0.001$; Apr vs. Oct, $p<0.05$).

Testicular diameter peaked in April (Figure 6-1), being significantly greater than the five months, August-November and January (Dunn's post hoc test: Apr vs. Oct & Jan, $p<0.01$; $p<0.001$ in all other cases). February-March and May-June were also associated with relatively high testicular diameters and were significantly higher than some of the months linked to lowest testicular diameters (Dunn's post hoc test: Feb-Mar vs. Aug & Sept, $p<0.01$; Feb-Mar vs. Nov, $p<0.001$; May-June vs. Nov, $p<0.01$).

6.2.1.3 Effect of Age

The dimensions of the testes, weight (Figure 6-2), and diameter (Figure 6-3) varied significantly with age (ANOVA, testicular weight: $F=20.085$; d.f.=2; $p<0.001$; Kruskall Wallis, testicular diameter: $\chi^2=13.969$; d.f.=2; $p=0.001$). Testicular weight in yearling was

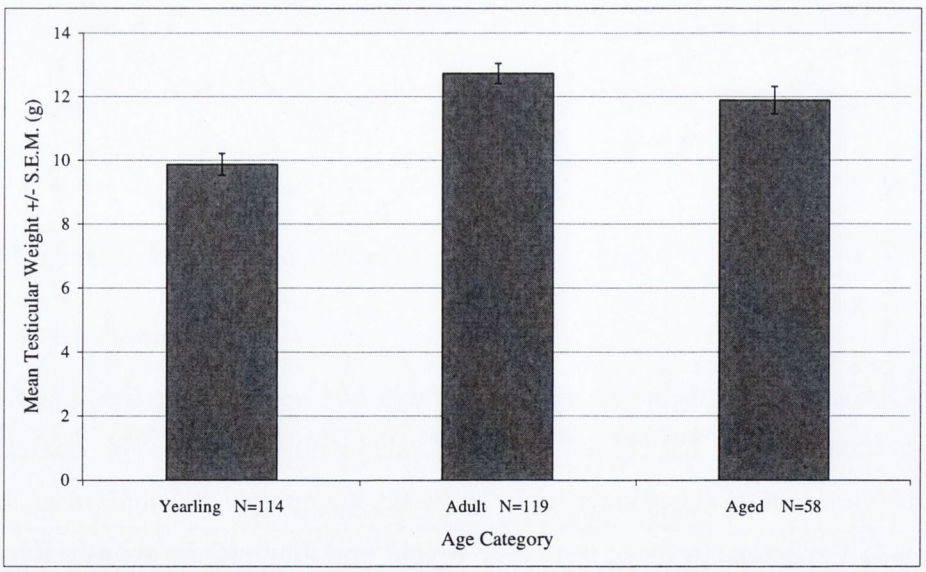

Figure 6-2: Mean testicular weight for the three age categories. Error bars represent standard error of the mean.

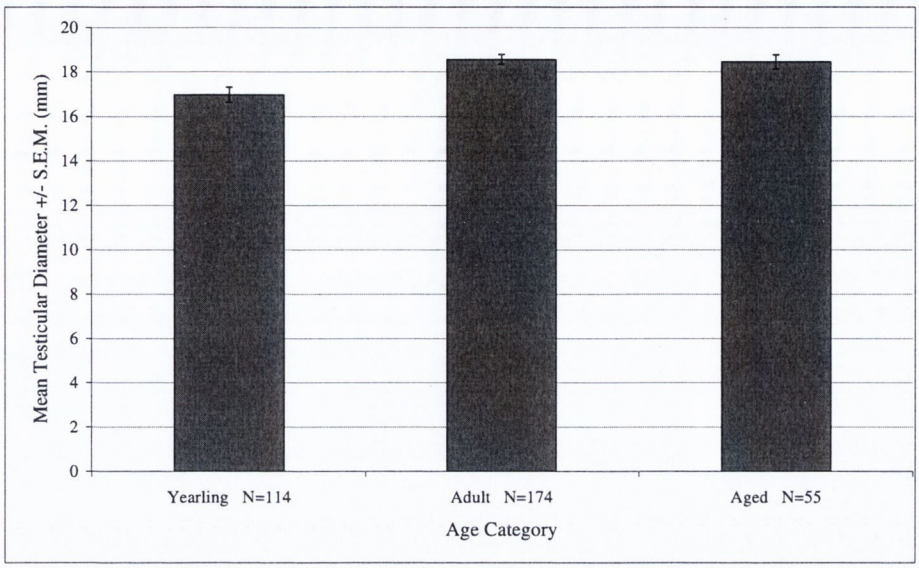

Figure 6-3: Mean testicular diameter for the three age categories. Error bars represent standard error of mean.

significantly lower than both adult and aged individuals (Figure 6-2) (Bonferroni post hoc test: $p<0.001$ in all cases). Adult badgers had significantly greater testicular diameters than yearlings (Figure 6-3) (Bonferroni post hoc test: $p<0.001$).

6.2.2 Seminal Vesicle Weight

6.2.2.1 Description

The weight of the seminal vesicle was recorded for each individual providing a sample size of N=287. The data were not normally distributed and therefore non-parametric methods of analysis were used.

6.2.2.2 Effect of time of year

Seminal vesicle weight varied little during the breading year; however it did increase to a peak in October (Figure 6-4). Variation between individuals also increased as the year progressed. Seminal vesicle weight varied significantly by month (Kruskall Wallis: $\chi^2=39.112$; d.f.=11; $p<0.001$); with the weights during the October peak being

significantly greater than weights in March, May and December (Dunn's post hoc test: $p<0.01$ in all cases).

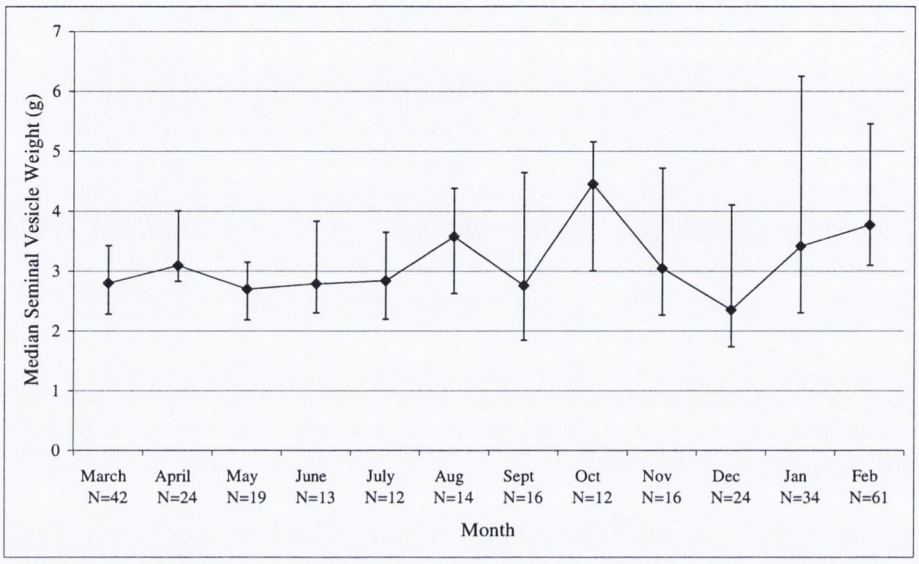

Figure 6-4: Variations in median seminal vesicle weight by season. Error bars represent the inter-quartile range.

6.2.2.3 Effect of age

Seminal vesicle weight varied significantly with age (Kruskall Wallis: $\chi^2=10.247$; d.f.=2; $p=0.006$) (Figure 6-5). There was a gradual increase in weight with increasing age, with aged badgers having the highest weights compared to the other two age categories. However, seminal vesicle weights for aged badgers were only significantly higher than yearlings (Dunn's post hoc test: $p<0.01$).

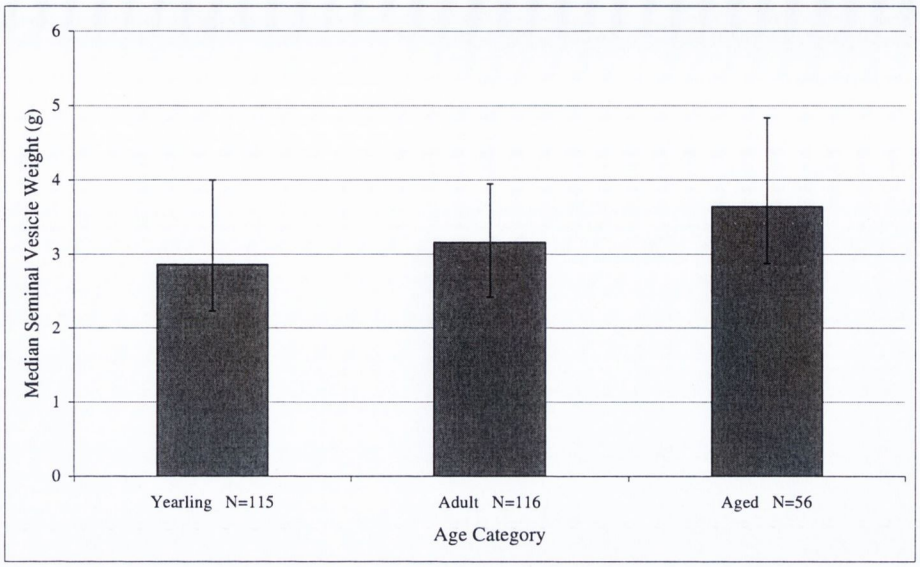

Figure 6-5: Median seminal vesicle weight for the three age categories. Error bars represent the inter-quartile range.

6.2.3 Circulating Testosterone

6.2.3.1 Description

The level of circulating testosterone was recorded for each individual. The extreme outliers were removed (cut-off: <–1.892 and >3.635pg/ul, n=2; for criteria see Section 2.5) which provided a sample size of N=252. The distribution of the data set was normalised by natural log transformation, allowing for the use of parametric statistical analysis. Where circulating testosterone levels were correlated with other parameters, the original non-transformed data set (excluding extreme outliers) was used along with non-parametric methods of statistical analysis.

6.2.3.2 Effect of time of year

Over the year testosterone levels varied widely with a decline from March to November and a peak covering December-January. A large peak in September interrupted the otherwise continuous decline in the first 10 months of the breeding year and gave the impression of a marked trough in October-November. Circulating testosterone levels

varied significantly by month (ANOVA: F=4.628; d.f.=11; p<0.001). In each month there was a high degree of variation between individuals (Figure 6-6).

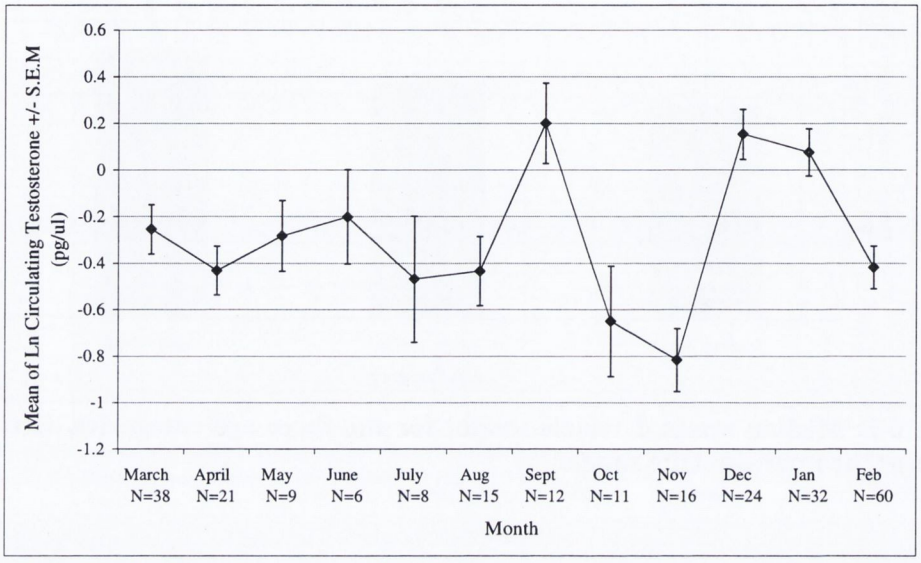

Figure 6-6: Variations in mean circulating testosterone by month. Error bars represent standard error of mean.

The peak in testosterone in September was significantly higher than in November (Bonferroni post hoc test: p<0.01) (Figure 6-6). December levels were significantly higher than the low levels in October-November and in February; levels in January were significantly higher than November and February (Bonferroni post hoc test: Dec vs. Nov & Jan vs. Nov, p<0.001; p<0.05 in all other cases).

6.2.3.3 Effect of age

Adult badgers had the lowest circulating testosterone levels of the three age categories with yearling and aged badgers having similar levels. However, circulating testosterone did not significantly vary with age (ANOVA: F=0.442; d.f.=2; p=0.643) (Figure 6-7).

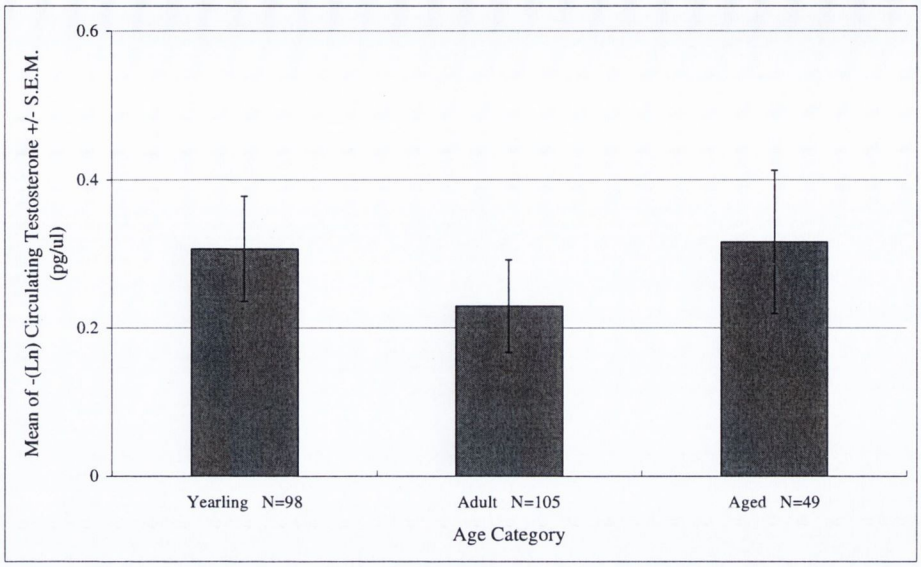

Figure 6-7: Mean circulating testosterone levels for the three age categories. Error bars represent standard error of mean.

6.2.3.4 Relationship of circulating testosterone to testicular weight

Although there was a trend for increasing testosterone levels with increasing testicular weight (Figure 6-8) the correlation was not significant (Spearman rank-order correlation: r_s=0.105; N=251; p=0.097).

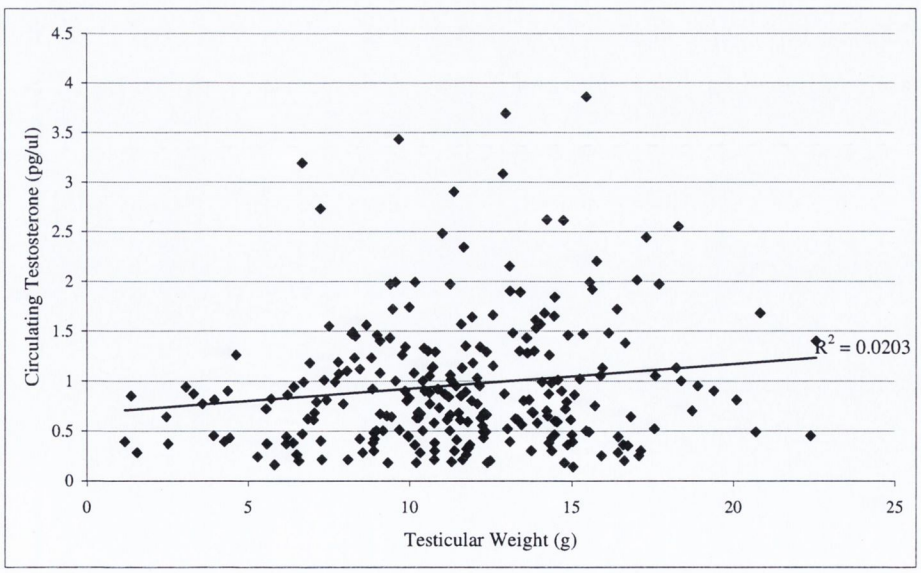

Figure 6-8: Distribution of circulating testosterone levels related to testicular weight for each badger.

6.2.4 Spermatogenesis

6.2.4.1 Description

Spermatozoa were identified in the seminiferous tubules of the testes (testicular spermatozoa) and the ducts of the cauda epididymides (epididymal spermatozoa). Abundance scores were assigned to each individual, on a scale of 0-4. It was possible to score 287 individuals for testicular spermatozoa and 291 individuals for epididymal spermatozoa abundance. Both data sets were not normally distributed; therefore non-parametric methods of analysis were used.

6.2.4.2 Proportion of males with spermatozoa present

Spermatozoa were present in the testes and the epididymides of at least some males during all months of the breeding year (Figure 6-9). Generally, the monthly proportions with spermatozoa present in the testes were similar to those with spermatozoa present in the epididymides. However, where discrepancies occurred, proportions were higher for spermatozoa in the testes.

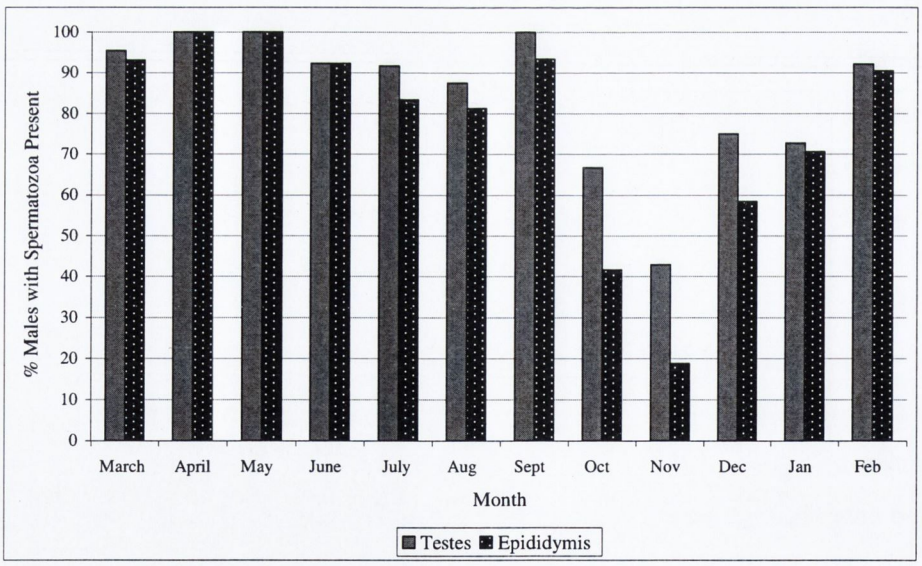

Figure 6-9: Proportion of males, as a percentage of the sample population, to have spermatozoa present in the testis and the epididymis.

For the majority of months, a high proportion of males had both testicular spermatozoa and epididymal spermatozoa present, fluctuating between 80% and 100% from February to September. A trough in both proportions occurred from October to January with November having the lowest values. The reduction in proportions was greater for epididymal spermatozoa, declining to 18.75% in November. No one age group was responsible for the lowest proportions during October and November (October: testicular spermatozoa (66.67%) yearlings=37.5%, adults=37.5%, aged=25%; epididymal spermatozoa (41.67%) yearlings=40%, adults=20%, aged=40%; November: testicular spermatozoa (42.86%) yearlings=33.33%, adults=50%, aged=16.67%; epididymal spermatozoa (18.75%) yearlings=33.33%, adult=33.33%, aged=33.33%).

6.2.4.3 Effect of time of year on spermatozoa abundance

Abundance scores for testicular spermatozoa remained constant for the eight months from February to September (Figure 6-10). This was followed by a trough of four months from October to January, suggesting a period of reduced sexual activity. November was associated with the lowest abundance scores of the year.

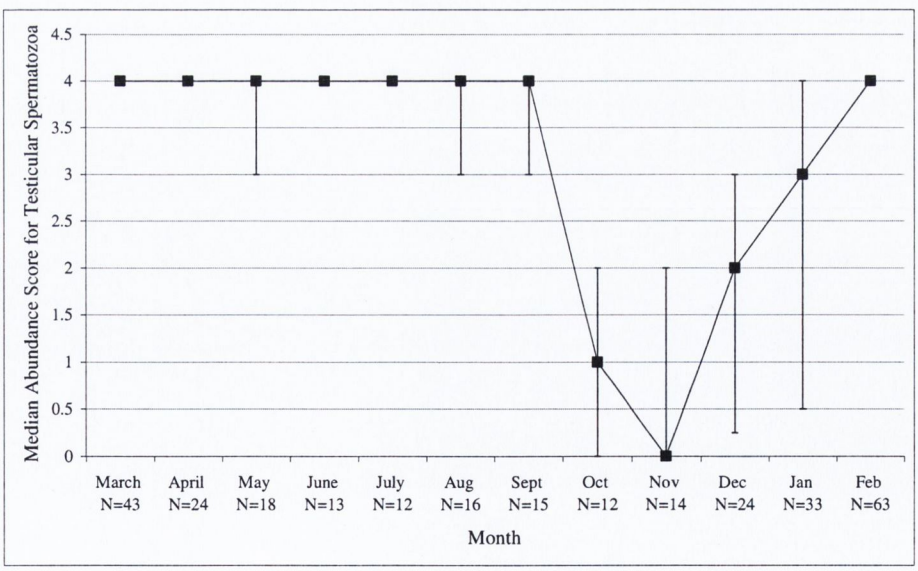

Figure 6-10: Variations in median abundance scores for testicular spermatozoa by month. Error bars represent the inter-quartile range.

Abundance scores for testicular spermatozoa varied significantly with month (Kruskall Wallis: χ^2=124.20; d.f.=11; p<0.001). The period of October to December, which was associated with a reduction in abundance scores (Figure 6-10), had significantly lower scores than the eight months from February to September (Dunn's post hoc test: Oct vs. May & June, p<0.01; Oct vs. Aug & Sept, p<0.05; Dec vs. May & June; p<0.01; Dec vs. Aug & Sept, p<0.05; p<0.001 in all other cases).

Variation between individuals increased during the trough in abundance scores (October-December). Although the trend suggested that active spermatogenesis was reduced (October) or absent (November), there was a proportion of individuals who had abundance scores suggestive of active spermatogenesis during the months of October-November (53.85%, N=26). During these months 33.33% of yearlings (N=15) displayed active spermatogenesis, 85.71% of adults (N=7) and 75% of aged individuals (N=4).

Abundance scores for epididymal spermatozoa followed similar annual trends (Figure 6-11) to scores for testicular spermatozoa. Abundance scores remained reasonably constant during the year with a trough occurring during the three months of October to December (Figure 6-11), which further supports the suggestion of reduced sexual activity during this period. The increase in abundance scores following the decline in October-November was steeper for epididymal spermatozoa scores than testicular scores (Figure 6-11 vs. Figure 6-10).

There was a significant variation in monthly abundance scores for epididymal spermatozoa (Kruskall Wallis: χ^2=81.216; d.f.=11; p<0.001). October and November, which were associated with the lowest abundance scores, were significantly lower than 8 months (January-May and July-September) and 9 months (January-September), respectively (Dunn's post hoc test: Oct vs. Jan, July & Aug, p<0.05; Oct vs. Feb & Apr, p<0.01; Nov vs. June, p<0.05; Nov vs. Jan, April, July & Aug, p<0.01; p<0.001 in all other cases). December, which was also part of the trough, was significantly lower than four months, February, March, May and September (Dunn's post hoc test: Dec vs. Mar, p<0.01; Dec vs. May, p<0.001; p<0.05 in all other cases).

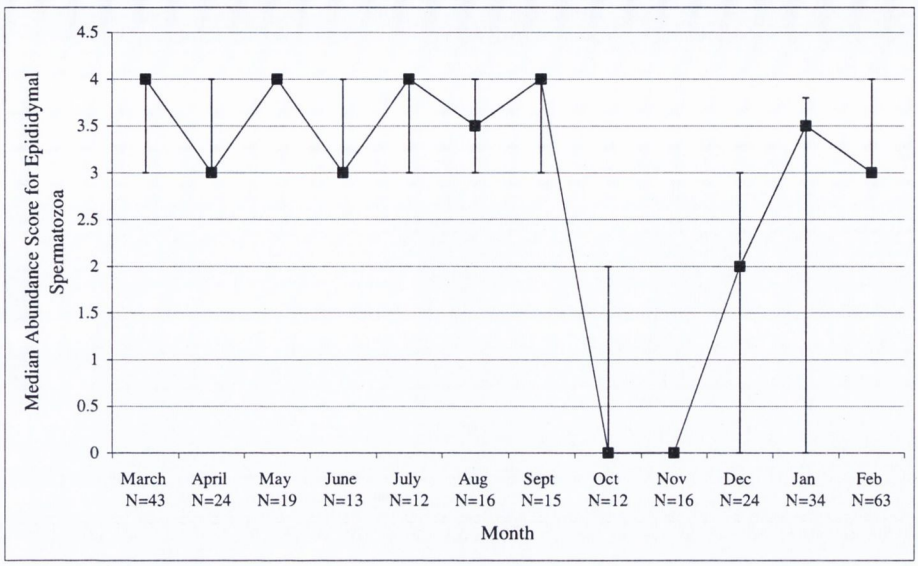

Figure 6-11: Variations in median abundance scores for epididymal spermatozoa by month. Error bars represent the inter-quartile range.

Although the trend suggested that epididymal spermatozoa was absent during October-November, there was a small proportion of individuals for whom epididymal spermatozoa remained present (28.57%, N=26). During these months 20% of yearlings (N=15) had spermatozoa present in their epididymides, 28.57% of adults (N=7) and 50% of aged individuals (N=6).

6.2.4.4 Effect of age on spermatozoa abundance

Despite having the same median as the two other age categories, there was a high degree of variation among abundance scores for testicular spermatozoa in yearlings (Figure 6-12). Compared to the other two age categories, yearlings had a high proportion of individuals with abundance scores of zero (yearlings, 30%; adults, 3%; aged, 2%). When tested, abundance scores varied significantly with age (Kruskall Wallis: χ^2=24.007; d.f.=2; p<0.001); with adult and aged badgers having significantly higher abundance scores compared to yearlings (Dunn's post hoc test: p<0.001).

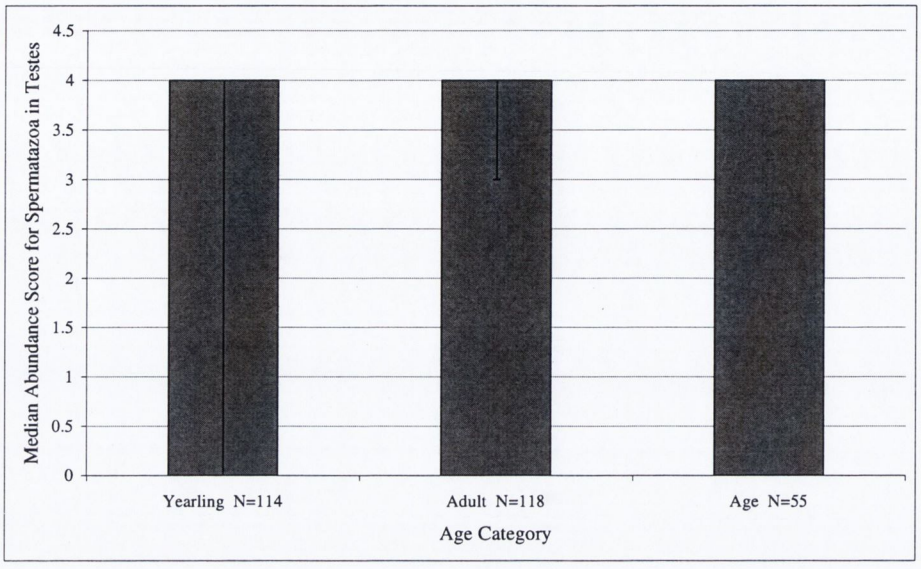

Figure 6-12: Median testicular spermatozoa abundance scores for the three age categories. Error bars represent the inter-quartile range.

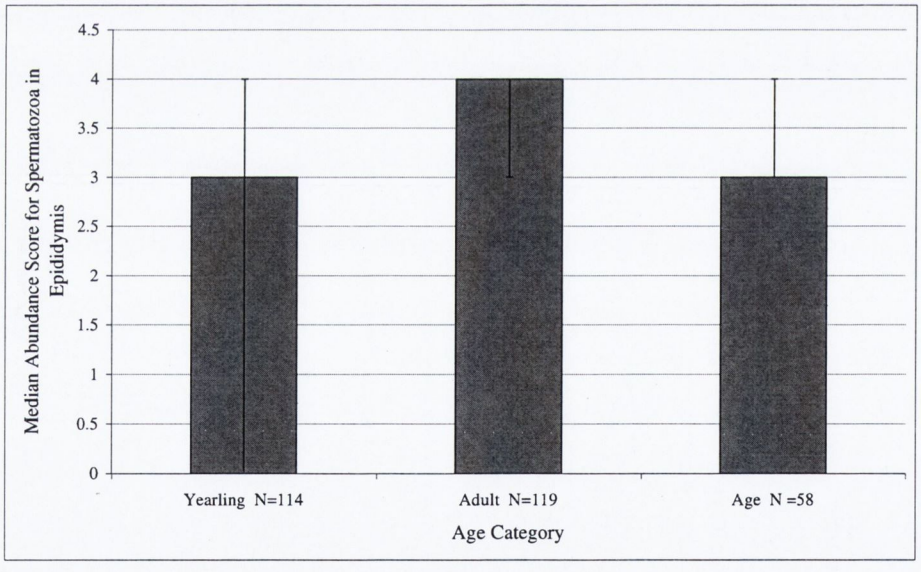

Figure 6-13: Median epididymal spermatozoa abundance scores for the three age categories. Error bars represent the inter-quartile range.

Adult badgers had the highest abundance scores for epididymal spermatozoa Figure 6-13. As with testicular abundance scores, there was high degree of variation among yearlings;

yearlings had a high proportion of individuals with abundance scores of zero compared to the other two age categories (yearlings, 34%; adults, 11%; aged, 7%). When tested abundance scores varied significantly with age (Kruskall Wallis: χ^2=18.211; d.f.=2; p<0.001); with adult and aged badgers having significantly higher abundance scores compared to yearlings (Dunn's post hoc test: yearling vs. adult, p<0.001; yearling vs. aged, p<0.01).

6.2.4.5 Relationship of abundance scores to testicular weight

There was a strong trend of increasing testicular weight with increasing abundance scores for both testicular spermatozoa (Figure 6-14) and epididymal spermatozoa (Figure 6-15). Both of these parameters had significant positive correlations with testicular weight (Spearman rank-order correlation: testicular spermatozoa vs. testicular weight, r_s=0.589; N=286; p<0.001; epididymal spermatozoa vs. testicular weight, r_s=0.457; N=290; p<0.001) and high R^2 values. Suggesting, that 43% of the variation in spermatozoa abundance in the testis and 35% of abundance in the epididymis was related to changes in testicular weight.

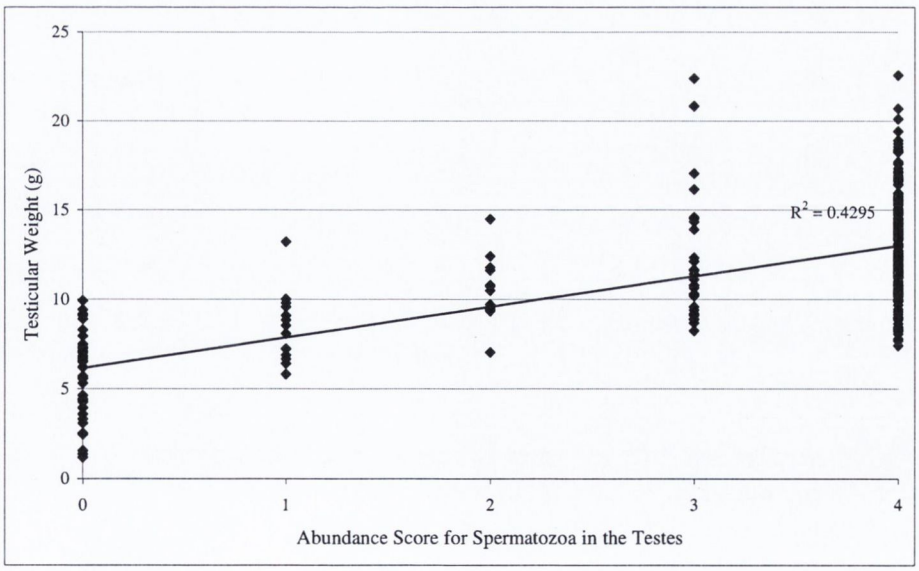

Figure 6-14: Distribution of abundance scores for testicular spermatozoa in relation to testicular weight for each badger.

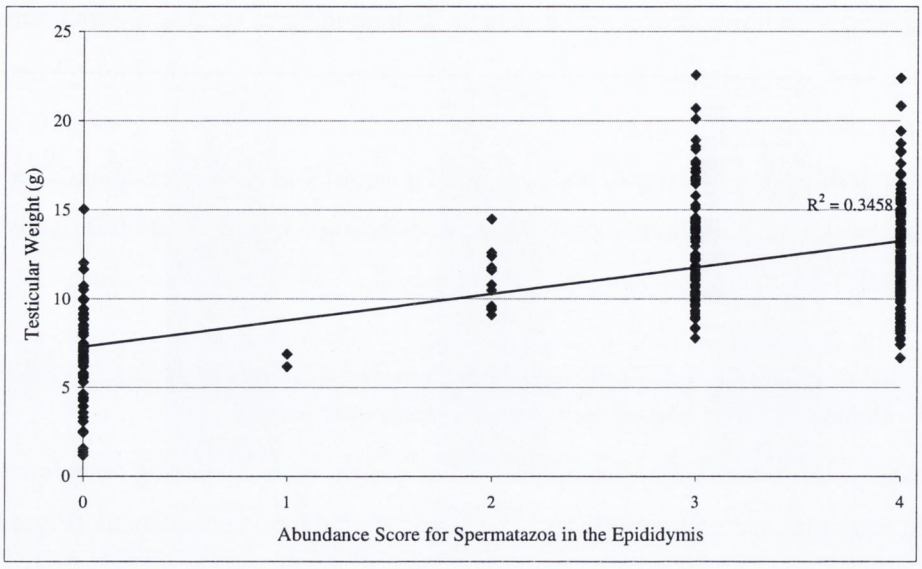

Figure 6-15: Distribution of abundance scores for epididymal spermatozoa in relation to testicular weight for each badger.

6.2.4.6 Relationship of abundance scores with circulating testosterone levels

There was little relationship between spermatozoa abundance in the testis and circulating testosterone levels (Figure 6-16), providing a non-significant correlation (Spearman rank-order correlation: r_s=0.069; N=240; p=0.280).

There was a slight, but significant, positive trend in the correlation of spermatozoa abundance in the epididymis and circulating testosterone levels (Figure 6-17) (Spearman rank-order correlation: r_s=0.147; N=252; p=0.020) but a low R^2 value (R^2=0.0152). Therefore circulating testosterone did not have a meaningful association with abundance scores of epididymal spermatozoa

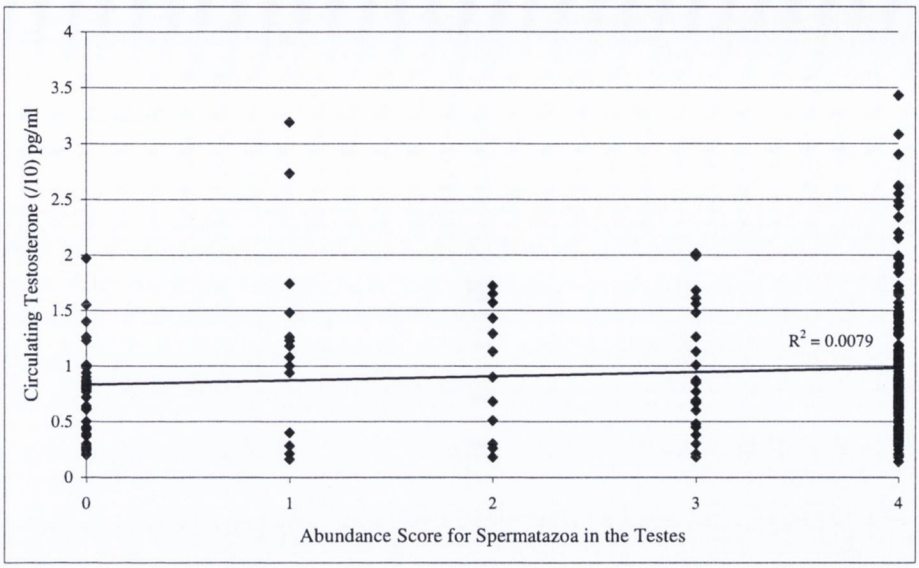

Figure 6-16: Distribution of abundance scores for testicular spermatozoa in relation to circulating testosterone levels.

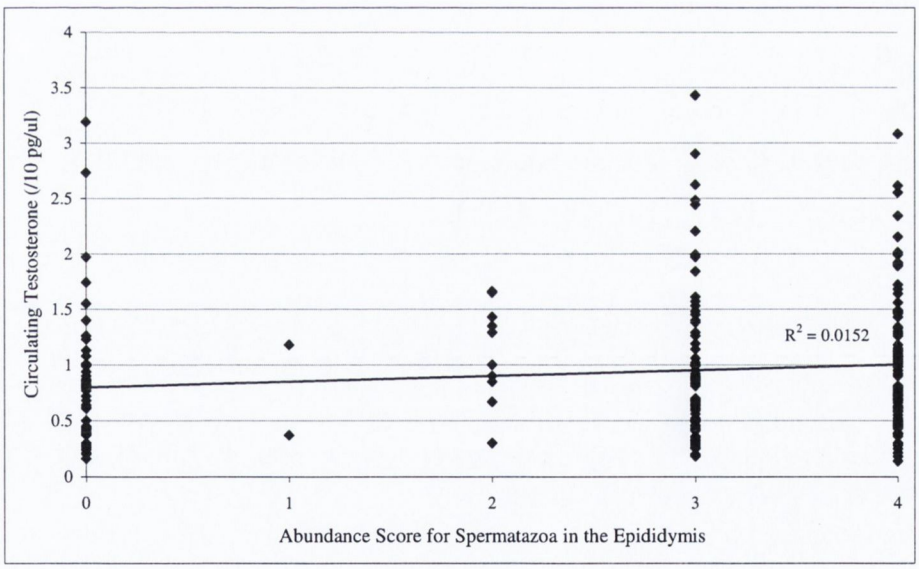

Figure 6-17: Distribution of abundance scores for epididymal spermatozoa in relation to circulating testosterone levels.

6.2.4.7 Comparison of sexually active and inactive males

Testicular weight, testosterone levels, body weight and subcutaneous fat depth were compared between sexually active and inactive males during the period of October-November (Figure 6-18). Males that remained sexually active during this period had significantly greater testicular weights than inactive males (t-test: t=-7.952; d.f.=18; p<0.001). However, there was no significant difference between sexually active and inactive males in testosterone levels (Mann Whitney: Z=-0.423; N=12, 7; p=0.672), body weight (t-test: t=-0.160; d.f.=13.14; p=0.875) or subcutaneous fat depth (Mann Whitney: Z=-1.362; N=12, 7; p=0.173).

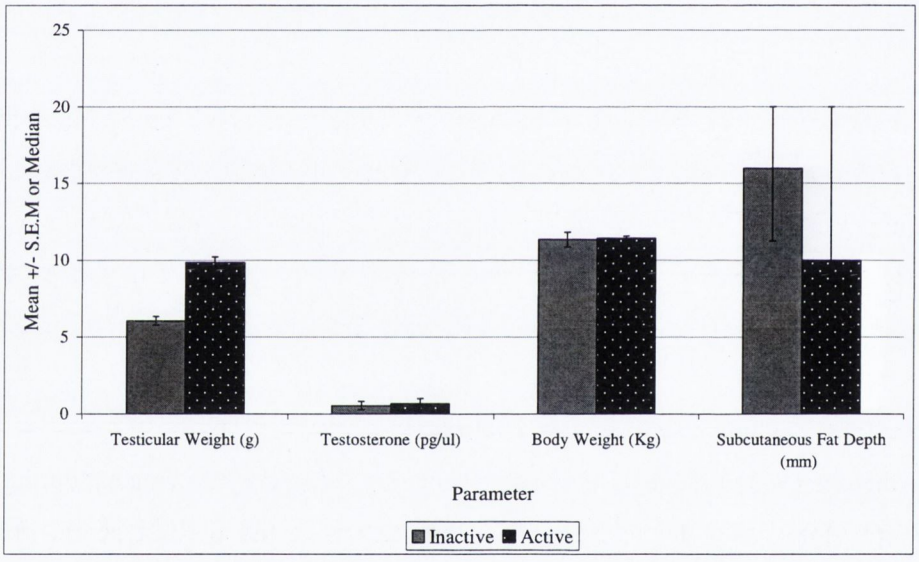

Figure 6-18: Mean testicular weight, median testosterone levels, mean body weight and median subcutaneous fat depth of males who were sexually inactive and active during the period of October-November. Error bars for testicular weight and body weight represent standard error of mean and inter-quartile range for testosterone levels and subcutaneous fat depth.

6.3 Summary

- The dimensions of the testes, testicular weight and diameter, decreased gradually during a protracted period from March to November. There was a distinct recovery in

testicular weight from December; with the peak in weight occurring in February. Testicular diameter also increased during the winter but less sharply than testicular weight; the peak in testicular diameter occurred later in April.

- Seminal vesicle weight varied little during the year but was highest in October. Contrary to: testicular weight, testosterone levels and spermatozoa abundance scores, all of which were at their lowest levels during October.

- Testosterone levels gradually declined from March to November, interrupted by a spike in September. This was followed by a rapid increase during winter to a peak in December-January.

- Spermatozoa were confirmed to be present in the testes and the epididymdes of a proportion of the male study population for each month throughout the year. The frequency of males with spermatozoa both in the testes and epididymides remained above 80% from February to September. Following which there was a trough in proportions during October to January; with the lowest frequencies occurring in October-November.

- Spermatogenesis in the testes (based on abundance scores) was greatest during March to September; with the mean remaining constant at the highest score. Following September there was a sharp decline during October-November, with abundance scores averaging zero in November. This was followed by an equally rapid recovery from December to February. Despite abundance scores being reduced during October-November, more than half of the male study population (53.85%, N=26) displayed active spermatogenesis during this period.

- Spermatozoa abundance in the epididymides fluctuated at a high level during the year from March to September. Consequently, there was a sharp decrease during October-November, where on average spermatozoa were absent from the epididymides. Abundance scores recovered during the subsequent months, returning to high levels in January-February. Despite the majority of individuals having abundance scores of

zero during October-November, there was a small proportion of individuals for whom epididymal spermatozoa remained present (28.57%, N=26).

- There was no correlation between testosterone levels and testes weight or spermatozoa abundance scores. However, spermatozoa abundance correlated well with testicular weight.

- All measures of male sexual activity: testicular dimensions, testosterone levels and the abundance score for spermatozoa volume were at their lowest levels during October-November.

- Males that remained sexually active during October-November had greater testicular weights than inactive males, but testosterone levels and body condition scores did not vary.

- There was no variation in testosterone levels between the three age classes but yearlings had the lowest testicular weights, seminal vesicle weights and abundance scores for testicular and epididymal spermatozoa

6.4 Discussion

Seasonal patterns of testicular activity, although varying within and between populations, generally coincide with the fertile period of females (Mead & Wright, 1983). The majority of adult males were involved in the breeding season which, for males, extends from February to September. Consistent with other studies, testicular weights rapidly increased in late winter to their highest values (February) and subsequently declined gradually until late autumn/early winter (Ahlund, 1980, Audy-Relexans, 1972, Page *et al.*, 1994, Woodroffe *et al.*, 1993). Testosterone levels were lowest in October-November but high in winter, which heralded the resumption of testicular activity in spring. The spike in testosterone levels in September may have been connected to resumption of hypothalmo-

pituitary activity, which is believed to occur in autumn for the badger (Audy *et al.*, 1985, Maurel *et al.*, 1984). Although testosterone levels declined steadily during the year (February-November), male fertility was initially not affected by this decline, as active spermatogenesis in the testes and spermatozoa volume in the epididymides was maximal during this period (February-September).

There was no variation in testosterone levels between the three age classes but yearlings had the lowest testicular weights, seminal vesicle weights and abundance scores for testicular and epididymal spermatozoa. This is suggestive of sexual immaturity. Males are believed to reach sexual maturity during their second year of life (12-24 months) (Ahlund, 1980, Neal & Harrison, 1958, Whelan & Hayden, 1993). Individuals assigned to the yearling age class were considered to be 12-24 months. The individuals within this group may have had their age overestimated or may not have reached sexual maturity until later in their second year of life.

The annual testosterone profile has previously been described as dropping significantly during July to November in the badger population in France (Audy *et al.*, 1985). This most likely reflects the breeding strategy adopted by males in that particular population, where endocrine testicular activity begins in January-February and ceases in July (Maurel *et al.*, 1984). In that population, testosterone levels were closely associated with testicular weight (Audy *et al.*, 1985). In the present study, there was no correlation between testosterone levels and testicular activity (testes weight and spermatozoa abundance scores), despite the parameters following similar annual trends. This may relate to the methodology used, as only one sample was taken from each badger, possibly providing an inaccurate measure of testosterone levels owing to the nature of its release. Testosterone is released in short bursts rather that being maintained at continual high concentration (Senger, 1997). However, spermatozoa abundance scores correlated well with testes weight, supporting the suggestion that testicular dimensions may be indicative of potential sexual activity (Ahlund, 1980, Page *et al.*, 1994)

In the present study, the peak in testicular activity occurred in February, prior to the post partum peak in female sexual activity, but continued for a considerable period beyond

what has previously been described for a population in France (Maurel *et al.*, 1984). This may be an adaptation by males in response to continued ovulations and sexual activity of females. It is possible that quantification of spermatogenesis may be a more reliable measure of sexual activity than testicular weight and therefore the duration of the male sexual activity may have previously been underestimated.

A small proportion of the males in the study population appeared to have adopted a different strategy of seasonal testicular activity. Whereas the majority enter a period of reduced sexual activity or quiescence during October and November, a small portion of the male population showed spermatogenesis and sexual activity throughout the year. Those males that remained sexually active during October-November had greater testicular weights, but testosterone levels and body condition scores did not vary from the males that entered a state of inactivity. It is interesting that the majority of males enter a phase of quiescence which is not associated with a loss in body condition. The low levels of testosterone during October-November corresponded with those described in the Wytham population (Woodroffe *et al.*, 1997). The lack of bite wounding within the study population suggests that territorial defence, mate guarding behaviour and intra-sexual competition for breeding opportunities may be significantly lower in the Irish population.

Continuous spermatogenesis throughout the year has been described in other populations based on smears of the caput epididymis e.g. Sweden and the south-west of England, (Ahlund, 1980, Page *et al.*, 1994). If continued female sexual activity occurs widely in badger populations, males that remain sexually active during the period when the majority of the population are sexually quiescent, would benefit from the increased mating opportunities and reduced competition. Mating late in the year can contribute to a relatively high proportion of fertilisations, as was seen for a population in the south-west of England. For this population, the late mating period in summer-autumn accounted for 29% of blastocysts produced (Cresswell *et al.*, 1992). In the present study maximal fertilisation rates were achieved in August which suggests that late matings may be of particular importance to the study population.

Different seasonal patterns of testicular activity have been described in badgers. A range of male sexual activity patterns are not just seen across large populations but between adjacent social groups (Woodroffe *et al.*, 1997) and across wide differences in latitudes. The pattern seen in the present study may be an example of a population effect. Differences in the seasonal activity, especially in the timing of the peak in activity are seen in Swedish badgers, where the lowest level of male sexual activity occurs earlier than in the British Isles, from August onwards (Ahlund, 1980).

Males commonly make forays into neighbouring territories which leads to a high proportion of extra-group paternity (Woodroffe *et al.*, 1993). Mate-guarding may be a feature of periods of high sexual activity stimulated by high levels of circulating testosterone. When testosterone levels are low there may be less aggression, less mate guarding, lower levels of permanent dispersion and higher levels of extra-group matings; as was described for Woodchester Park (Cheeseman *et al.*, 1988). Hence, males that remain sexually active at these times will have a reproductive advantage. In the south-west of England it was found that resident males experienced reduced fertility in the autumn, but those immigrants that remained sexually active had high testosterone levels, which remained until the end of October (Woodroffe *et al.*, 1997, Woodroffe & MacDonald, 1995b, Woodroffe *et al.*, 1993). Although these individuals may have had higher paternity levels than residents, they suffered greater reductions in body condition and had more recent bite wounds and broken canines (da Silva *et al.*, 1994, Woodroffe & MacDonald, 1995a, Woodroffe *et al.*, 1993), evidence of aggression from intra-group males defending oestrual females (Woodroffe & MacDonald, 1995a, Woodroffe *et al.*, 1997, Woodroffe *et al.*, 1993).

7 DISCUSSION

The studies described here have provided novel insights into the reproduction of badgers in Ireland. The combination of morphological, histological and hormonal data from both sexes gives a uniquely comprehensive picture of reproduction in this species; making it the most thorough study to have been undertaken so far. However, as the methodologies and sampling procedures used have not been previously employed in other studies, these insights may not be unique to Irish badgers.

Although the sample of the population examined in this study was derived from culling, the demographics of the sample may be representative of the population at large. The badger population in Ireland may be one of low-medium density consisting of small social groups, similar to those described in areas of Northern Ireland, England, Scotland and continental Europe (Northern Ireland & England: Feore & Montgomery, 1999, Scotland: Kruuk & Parish, 1987, Spain: Revilla & Palomares, 2002, Switzerland: Do Linh San *et* al., 2007, Poland: Kowalczyk *et al.* 2003). From the data it was not possible to determine what factors may influence group size and density in this population. However, it was apparent from comparisons with previous studies (O'Corry-Crowe *et al.*, 1993, Sleeman *et al.*, 2009), that badger group size may have declined over the past decades. The nationwide badger removal operation in Ireland conducted by DAFF has been more consistent since 2002-2003 and may have contributed, in part, to this reduction. The badger removal operation may also interfere with the demographic profile of the Irish badger population, causing it to vary between years. The sex ratio of the study population deviated from unity towards male bias; however, previous yearly sex ratios have reported unity or female biased sex ratios (O'Boyle, 1998, 1999, 2000, 2002, O'Boyle *et al.*, 2003) .

The lack of aggression observed within the study population suggests that there may be high levels of tolerance both, within and between social groups. This may result from small group sizes (Stewart *et al.*, 1997a), high levels of philopatry (Cheeseman *et al.*, 1988, Woodroffe *et al.*, 1993), inter-group relatedness (da Silva *et al.*, 1994), and/or relatively low levels of testosterone (Cheeseman *et al.*, 1988, Woodroffe *et al.*, 1997). Mate guarding behaviour may be abandoned due to high levels of philopatry (Woodroffe *et al.*, 1997) or an inability to monopolise paternity (Carpenter *et al.*, 2005, Woodroffe *et al.*, 1993). Although the results tentatively suggested that only one female successfully reproduced within a group, it is unlikely that this was as a result of reproductive suppression.

The studies showed that the basic nature and timing of reproduction in the badger was the same in Ireland as has been previously demonstrated elsewhere (Ahlund, 1980, Cresswell *et al.*, 1992, Neal & Harrison, 1958, Page *et al.*, 1994, Whelan & Hayden, 1993). However, there was an intriguing difference related to loss of reproductive potential. Failure to become fertilised and loss of blastocysts during the period of diapause, rather than losses later in the cycle (Cresswell *et al.*, 1992, Page *et al.*, 1994, Whelan & Hayden, 1993), were the greatest influence in reducing reproductive performance. Once blastocysts were maintained the majority of females completed the breeding cycle. In accordance with a previous Irish study (Whelan & Hayden, 1993), approximately 40% of sexually mature females bred successfully. Although this proportion was greater than that described for a high density population (Cresswell *et al.*, 1992), it was considerable lower than that of low density populations (Ahlund, 1980, Revilla *et al.*, 1999). This suggests that Ireland may be of an intermediate density or that factors, other than density, may influence the reproductive potential of this population.

Morphological and histological data obtained during the studies were able to provide strong support for females entering oestrus during the period of diapause and the potential for superfetation to occur, a theory that hitherto has been controversial. Observations of cyclic changes to the vaginal epithelium (Neal & Harrison, 1958), folliculogenesis (Harrison & Neal, 1956), and the presence of ova (Harrison & Neal, 1956, Page *et al.*, 1994) in successfully fertilised females have previously been described in a small number

of females. In the present study it was reported that 18% of fertilised females entered oestrus while possessing potentially viable blastocysts. Furthermore, although relatively low compared to a number of previous studies in Ireland and south-west England (Cresswell *et al.*, 1992, Whelan & Hayden, 1993), a proportion of fertilised females during September to December (7%) possessed blastocysts of two distinct sizes and a corresponding number of corpora lutea. Therefore, as with American mink, badgers may exhibit some level of superfetation, which may lead to greater opportunities for polyandry and improved female fitness (Yamaguchi *et al.*, 2006, Yamaguchi *et al.*, 2004). The long period of diapause exhibited by badgers would greatly facilitate such reproductive mechanisms (Yamaguchi *et al.*, 2006).

The presence of additional corpora lutea, although possibly resulting from failed fertilisations or loss of blastocysts, may provide support for an alternative theory first suggested by Harrison & Neal (1956). We propose that these additional corpora lutea may supplement or re-establish progesterone levels necessary for maintenance of the blastocyst, implantation and gestation (Bonnin *et al.*, 1978, Canivenc & Bonnin, 1981, Martinet *et al.*, 1981, Mead, 1981). The additional corpora lutea may arise from continued oestrus cycles while females are in diapause and/or from secondary follicles or non ovulated corpora lutea as was described for the mink (Mossman & Duke, 1973).

The seasonal pattern of testicular activity varied among the population, but generally coincided with the fertile period of females. The majority of adult males, in accordance with previous studies (Ahlund, 1980, Audy-Relexans, 1972, Page *et al.*, 1994, Woodroffe *et al.*, 1993), were involved in a breeding season which extended from February to September; maximal fertilisation rates in females were achieved in August. However, a small proportion of males showed spermatogenesis and sexual activity throughout the year. Males who remained sexually active had greater testicular weights, but testosterone levels and body conditions did not vary from males that entered a period of reduced sexual activity or quiescence. Continuous spermatogenesis throughout the year has been described in other populations (Ahlund, 1980, Page *et al.*, 1994) and may be associated with substantial benefits. Males that remain sexually active during the period when the majority of the population are sexually quiescent may potentially profit from increased

mating opportunities and reduced competition. That a proportion of females enter oestrus in November is further support for this breeding strategy.

A comparison of male and female badgers suggests that each has adopted a very different breeding strategy. The majority of males show a breeding pattern that is reminiscent of a seasonal breeder, with high fertility associated with the early oestrous cycles in the female and declining fertility for the remainder of the year. Males that remain fertile for longer periods may increase their chances of paternity during later matings. By contrast the female has adopted a strategy whereby they are lactating during a period of nutritional abundance, and cubs are also weaned during favourable conditions. This is achievable due to the long period of delay employed by this species. Badgers are polyoestrous, having continued oestrous cycles throughout the breeding year. This provides replacement or additional blastocysts, which increases the probability of successful implantation at the end of the period of diapause. Furthermore, continued oestrous cycles may increase the probability of superfeation and polyandry, leading to increased female fitness and possibly cub survival. This female strategy may also provide additional corpora lutea, which act as a source of progesterone, necessary for maintenance of the blastocyst, implantation and gestation. Failure at the fertilisation stage of the reproductive cycle was responsible for the greatest losses to reproductive potential; with approximately 40% of sexually mature females breeding successfully.

8 APPENDIX I

(i) Fixation

The samples were taken to the Veterinary Research Laboratory in Backweston where they were processed using an automated tissue processor (Make & Model: Sakura Tissue-Tek VIP). This machine dehydrates the sample through graded ethanol solutions and then replaces the ethanol with paraffin wax. The following timings were used:

70% Alcohol	1 hour
90% Alcohol	1 hour
Absolute Alcohol I	1 hour
Absolute Alcohol II	1 hour
Histoclear	1 hour
Histoclear/Paraffin wax mix	1 hour
Wax I	1 hour
Wax II	1 hour

(ii) Paraffin Wax Embedding and Sectioning

The embedding and sectioning were done by Marion Barrett at the Veterinary Research Laboratory and personally in Trinity College Dublin. The samples processed in Trinity were collected from the Veterinary research laboratory following dehydration. The samples which remained in their cassettes were grouped in jars and preserved in wax.

Prior to embedding in wax, the jars of cassettes were placed in an oven at 60°C (Make & Model: Gallenkamp Economy Incubator with Fan) for an hour to melt the wax and free

the cassettes. The tissues were embedded in paraffin wax using a histobank machine (Make & Model: Shandon Histocentre 2). Transverse sections were cut from the wax blocks at a thickness of 4μm using a microtome (Make & Model: Leica RM 2255). The sections were floated in 30% ethanol for approximately 5 minutes and then transferred to a warm water bath (~40℃) for a further 5 minutes to allow them to flatten completely. Using a clean labelled slide the sections were lifted onto the surface of the slide and left to dry. One section was taken from each wax block.

(iii) Staining – Haemotoxylin & Eosin

The dry slides were brought to the Veterinary Research Laboratory for staining with haematoxylin and eosin (H&E) and mounted in neutral xylene soluble mounting medium (60% xylene & butyl acrylate) using an automated stainer integrated with a coverslipping machine (Make & Model: Leica Multistainer ST 5020, integrated with Leica CV 5030 coverslipper). The following timings were used for the H&E stain:

Histoclear	1-3mins
Absolute Alcohol I	1-2mins
Absolute Alcohol II	1-2mins
90% Alcohol	2mins
70% Alcohol	2mins
Haemotoxylin Stain	2-10mins
Scott's Tap Water	1-4mins
Eosin Stain	2mins
70% Alcohol	1-2mins
90% Alcohol	1-2mins
Absolute Alcohol	2mins
Histoclear	2-5mins

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