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REVIEW ARTICLE

Deep Uterine Insemination of Cattle: A Fruitful Way Forward with Smaller Numbers of Spermatozoa

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Hunter RHF, Greve T: Deep uterine insemination of cattle: A fruitful way forward with smaller numbers of spermatozoa. Acta. vet. scand. 1998, 39, 149-163. – After describing the site of fertilisation and that of the functional sperm reservoir in the female tract, proposals are made concerning a modified site of sperm deposition in cattle.By means of a deep pre-ovulatory insemination into the ipsilateral uterine horn, the chances should be raised of establishing viable spermatozoa in the isthmus where they would undergo a form of physiological encapsulation and storage. Release and activation of such spermatozoa would be prompted by imminent ovulation.
Potential advantages of this approach include those of raising the overall fertility of genetically valuable bulls whose non-return rates are sub-optimal; reducing the number of spermatozoa in each insemination dose; using effectively the limited numbers of sex-selected sperm cells (X and Y chromosome bearing spermatozoa) currently available from flow cytometry. Putative disadvantages might include rectal palpation of the ovaries to locate the pre-ovulatory follicle; perforation of the uterine wall by the deep insemination catheter; risk of polyspermic fertilisation; and the inappropriateness of the technique for non-clinically qualified inseminators. Each of these reservations is responded to in a rational manner. Given a change of attitude, a modified technique of insemination would be feasible under commercial conditions and might give a welcome boost to a sagging artificial insemination industry.

uterus; cow; fertilisation; sperm reservoir; Fallopian tube; isthmus; polyspermy; glycoproteins.

Introduction

Despite the long established and widely practised technique of artificial insemination – undoubtedly the oldest and most successful of reproductive technologies applied to animal reproduction – specific interactions between mammalian spermatozoa and the female genital tract remain imperfectly understood. The following essay summarises our current view of the progress of bull spermatozoa in the female tract relative to the time of ovulation. Drawing on this physiological description, modifications are proposed to conventional procedures of insemination. In essence, these involve deposition of a sperm suspension towards the distal tip (ovarian extremity) of the appropriate uterine horn. As it is more than 50 years since the first commercial centres for artificial insemination of cattle were organised in Western Europe, it is none too soon for serious appraisal of traditional approaches.

The immediate question is, of course, whether a modified technique of insemination could lead to improved fertility, that is increased conception or non-return rates in a proportion of cows and heifers so treated. The answer is almost certainly 'yes', although one that will be influenced by the individual bulls under test. Putting instances of pathology to one side, the overall premise will be that a sub-optimal conception rate in cattle inseminated at the correct time is more likely to be due to a dearth or absence of viable spermatozoa at the site of fertilisation than to an excess. Assuming a sufficient number of cells is introduced at insemination, a dearth or absence of competent spermatozoa would be a reflection of (a) either inadequate sperm survival in the female tract or (b) failure to establish a functional sperm reservoir, or a combination of both.

A logical start to this essay is to clarify the actual *site of fertilisation* and likewise that of the *functional sperm reservoir,* since these become reference points in the subsequent discussion. Brief mention of the viable lifespan of a secondary oocyte in the Fallopian tube is also necessary. Background literature would include classical texts such as *Salisbury et al.* (1978), *McDonald* (1980), *Gordon* (1983, 1996), *Cupps* (1991) and *Hafez* (1993), and various published dissertations (eg. *Greve* 1981, *Hyttel* 1988, *Larsson* 1988).

Site of fertilisation

The region of the female tract in which a mammalian egg is fertilised is the ampullary-isthmic junction of the Fallopian tube (Fig. 1; *Austin* 1961, *Blandau* 1969, *Yanagimachi* 1994). Aided by cumulus cells attached to at least a portion of the zona pellucida (*Lorton & First* 1979, *Hyttel* 1988), the newly ovulated secondary oocyte is rapidly displaced from the collapsed Graafian follicle to the site of fertilisation. Egg transport to the ampullary-isthmic junction takes some 9–13 min in rabbits (*Harper* 1961) and no more than 30–45 min in pigs (*Oxenreider & Day* 1965, *Hunter* 1974). There is no reason to suppose that it takes significantly longer in cattle although there are no direct measurements *in vivo.* However, even if a competent spermatozoon were to reach the egg's surface at some point during transit down the ampulla, there would be insufficient time for fertilisation to be achieved before the egg reaches the ampullary-isthmic junction. Thus, if fertilisation is to occur at all, it will be in this portion of the duct system, following which there is a relatively prolonged residence of the zygote in the same region before entering the isthmus.

The viable lifespan of a cow egg – the period of time after ovulation during which it can be fertilised without anomalies – is still not known with precision. Nor is it known whether this varies from animal to animal or even between subsequent ovulations in the same animal. Various lines of evidence indicate a lifespan of approximately 8 h, after which sperm penetration of the vitellus may give seemingly normal fertilisation but not a normally developing embryo. In other words, apoptotic events may already have been instigated in the secondary oocyte by the time of sperm penetration (see *Casida* 1950, *Austin* 1970, *Hunter & Greve* 1997). Whether so-called hardening of the zona pellucida in the Fallopian tube acts to prevent penetration by bull spermatozoa also remains to be clarified. But the lifespan of 20–24 h suggested by *Thibault* (1967) for cow eggs appears not to be meaningful and was not derived from physiological experiments.

Functional sperm reservoir

Semen is ejaculated deep into the vagina during mating, and almost immediately a suspension of spermatozoa bathes the mucous interface at the external cervical os; this enables motile

cells to gain the canal very soon thereafter. Large numbers of spermatozoa in the vaginal pool are voided from the tract, and the greatest reserves of spermatozoa remaining are located in the cervix within 6–8 h of mating. As revealed by conventional light microscopy, the crypts and also the mucus-filled cervical canal contain substantial numbers of spermatozoa (*Laing* 1945, *Mattner* 1966 & 1968, *Thibault* 1973), with the majority of those in the crypts having their heads orientated towards the epithelium. This disposition led researchers in the 1960s and 1970s to view the ruminant cervix as the principal pre-ovulatory sperm reservoir (see *Mattner* 1966, *Gibbons* 1969, *Robinson* 1973 *Thibault et al.* 1973) and, quantitatively speaking, this remains true. However, it is not the functional sperm reservoir – the one of relevance to this essay – nor is there any evidence that spermatozoa established in the cervical crypts can contribute directly to the events of fertilisation. *Mullins & Saacke* (1989) suggested that such spermatozoa progress to the uterus within longitudinal grooves and channels in the cervical tissues, but this remains hypothesis.

The reservoir from which the fertilising spermatozoon is released close to the time of ovulation is in the caudal region of the Fallopian tube isthmus. Details of this functional sperm reservoir in cattle can be deduced from histological (*Thibault* 1973, *Thibault et al.* 1973, *Larsson* 1988), surgical (*Hunter & Wilmut* 1982 & 1984) and scanning electron microscopic studies (*Hyttel* 1988, *Hunter et al.* 1991, *Pollard et al.* 1991). Viable spermatozoa that have gained the functional sperm reservoir after mating early in oestrus are arrested during the pre-ovulatory interval by binding of the sperm head to organelles of the isthmus epithelium, cilia or microvilli or both, until release of the egg is imminent. There is a striking contrast between highly active spermatozoa suspended in fluid of the uterine lumen and strongly suppressed spermatozoa, bound to the isthmus epithelium before ovulation. Even though the binding reaction between the rostral portion of the sperm head and cells of the endosalpinx appears to be avid, and seemingly involves fucose and a fucose-containing ligand on the epithelium (*Lefebvre et al.* 1997), reduced sperm motility

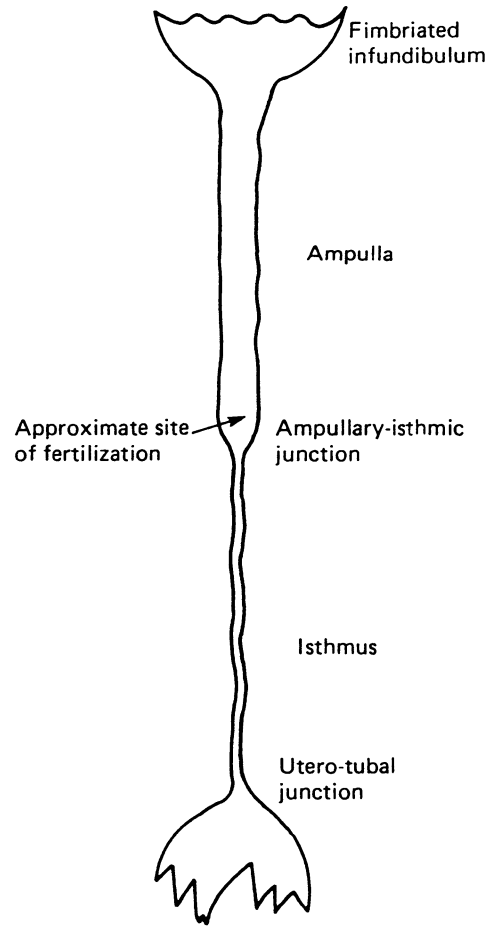

Figure 1. Linear representation of a bovine Fallopian tube to depict the thin-walled ampulla leading into the strongly muscular isthmus. The site of fertilisation – that is of successful gamete fusion – is in the region where ampulla and isthmus merge.

may nonetheless be necessary to enable the cell adhesion reaction to be initiated and to function effectively during the pre-ovulatory interval.

Shortly before ovulation, spermatozoa become activated and released in tightly controlled numbers, indicating a discrete influence on sperm motility and the binding reaction. Although not demonstrated in the cow (*Hyttel et al.* 1991), local endocrine control of the reservoir portion of the porcine Fallopian tube has been shown to originate from Graafian follicles close to and on the point of ovulation, and to be transmitted principally by a counter-current vascular exchange mechanism in the adjacent ovarian pedicle (*Hunter et al.* 1983). Hence the structure that will shed the female gamete as a secondary oocyte coordinates a controlled release of male gametes from storage in the caudal isthmus. Initial sperm:egg ratios at the site of fertilisation would be close to unity. Studies failing to endorse this aspect in cattle may have missed a critical point. The local ovarian (ipsilateral) influence on sperm release and initial sperm:egg ratios should be detectable close to the time of ovulation, but would soon be masked by systemic influences of ovarian hormones.

Viscous glycoprotein secretions seen as a fine mucus in the caudal isthmus during oestrus may have a special rôle to play in sperm storage. They could act not only to diminish sperm motility and stabilise sperm membranes but also to prevent free access of uterine fluids and Fallopian tube ampullary fluids into the reservoir region where they might stimulate sperm motility. Viable spermatozoa stored within the glycoprotein secretion of the isthmus undergo a kind of physiological sperm encapsulation and are protected from polymorphonuclear leucocytes. Gradual release from such encapsulation would be prompted by endocrine changes close to ovulation time, which may be in contrast to events surrounding in vitro sperm encapsulation using a synthetic medium before insemination (*Nebel et al.* 1993, *Vishwanath et al.* 1997).

Having defined the two critical regions of the Fallopian tube in the light of relevant research, especially in domestic farm animals, there is now a need to argue why *deep uterine insemination* could be beneficial in cattle. The most immediate justification would be that an appropriately-timed insemination before ovulation should increase the chances of: (1) establishing a functional sperm reservoir in the isthmus; (2) enabling sperm binding reactions with the endosalpinx; and (3) permitting consequent molecular modification and remodelling of the sperm head membranes as a preliminary to successful fertilisation.

There have been previous trials of relatively deep insemination in cattle (see *Olds* 1978), but the results were frequently unconvincing until, most notably, those of *Senger et al.* (1988). However, results of the early trials may have been confounded by (1) a variable timing of insemination relative to ovulation, and (2) the customary withdrawal of the pipette or straw during insemination resulting in a non-specific site of deposition of the sperm suspension.

Potential advantages of deep insemination

Overall fertility

A principal justification for encouraging deep cornual insemination could be to try to improve the fertility of valuable but sub-optimal bulls as monitored by enhanced non-return rates. In reality, the procedure would aim to increase the incidence of eggs fertilised. The rationale has already been presented that failure of fertilisation is more likely to be associated with a shortage of competent spermatozoa in the vicinity of the egg than with an excess. Using appropriate numbers of viable sperm cells, insemination closer to the functional sperm reservoir than is routine should therefore increase the incidence

of successful fertilisation. Even if this figure were to be raised by only 3%–5%, the impact on national calving statistics would be appreciable, although less than the suggested figures of 3%–5% due to prenatal mortality. Under Western conditions of agriculture, actual calving rates seldom exceed 55%, even when inseminating sperm suspensions from bulls judged to be the most fertile (*Hawk* 1979).

Bulls desirable for various genetic traits but with a fertility ranking lower than those in the top category might show improved non-return rates. Quite apart from the increased probability of establishing an isthmus sperm reservoir with deep insemination, spermatozoa would not be exposed first to the lumen of the uterine body and then to the full extent of a uterine horn. There is good evidence from other domestic species that spermatozoa of certain stud males are vulnerable to the uterine environment after freezing and thawing (*Polge et al.* 1970). The sperm cell plasma membrane is seemingly modified and rendered more fragile by the cryotechnology. This might be especially so for certain low fertility bulls. Deep insemination would clearly reduce the extent of uterine exposure – exposure to the endometrium, to uterine luminal fluids, and to the rapidly infiltrated population of polymorphonuclear leucocytes – for at least a proportion of those spermatozoa subsequently entering the Fallopian tubes. The advocated approach should therefore help to establish a higher proportion of undamaged spermatozoa still capable of fertilising an egg. It may be relevant to add that deep insemination into the sheep uterus by means of a laparoscopic approach has yielded valuable results with frozen-thawed ram spermatozoa (*McKelvey et al.* 1985, *Robinson et al.* 1989).

Reduced number of spermatozoa inseminated

A second aspect concerns the 'sperm gradient' – a progressively reduced number of spermatozoa that can be demonstrated along the female tract after natural mating (Fig. 2). As a consequence of this gradient, significantly fewer spermatozoa are counted at the tip of a uterine horn than in the uterine body (*Austin* 1965, *Thibault* 1973, *Larsson* 1988). To mimic this situation, it would be prudent to introduce a reduced number of spermatozoa (*eg.* ×100 reduction) close to the utero-tubal junction. Indeed, if working with only small volumes of a sperm suspension (*ie.* 0.1–0.25 ml), this would seem the most sensible site of deposition.

Apart from the economic advantages of reducing the number of spermatozoa introduced in commercial insemination, the technique might also become attractive when trying to improve conception from oligospermic bulls possessing valuable genetic traits.

Sperm plasma membrane proteins or glycoproteins are apparently modified sequentially after mating during progression along the female tract, as is doubtless also the case after artificial insemination. Loss or displacement of male membrane-bound glycoproteins may be accompanied by addition and/or substitution of female (uterine and Fallopian tube) glycoproteins (Fig. 3). If there is any validity in such speculation, then a clearer understanding of these substitutions and/or interactions and of the specific molecules involved in the female tract might offer a means of exploiting bulls whose semen gives poor results upon freeze-thaw procedures. When working with reduced numbers of spermatozoa, as suggested above, a controlled addition of appropriate macromolecules to the inseminate might be beneficial.

There is one other aspect to these speculations that deserves consideration. The putative male × female tract interactions in terms of modifications to the sperm cell that bestow full fertilising ability may offer a valuable insight into why laboratory estimates of semen quality to predict field-scale fertility data (non-return

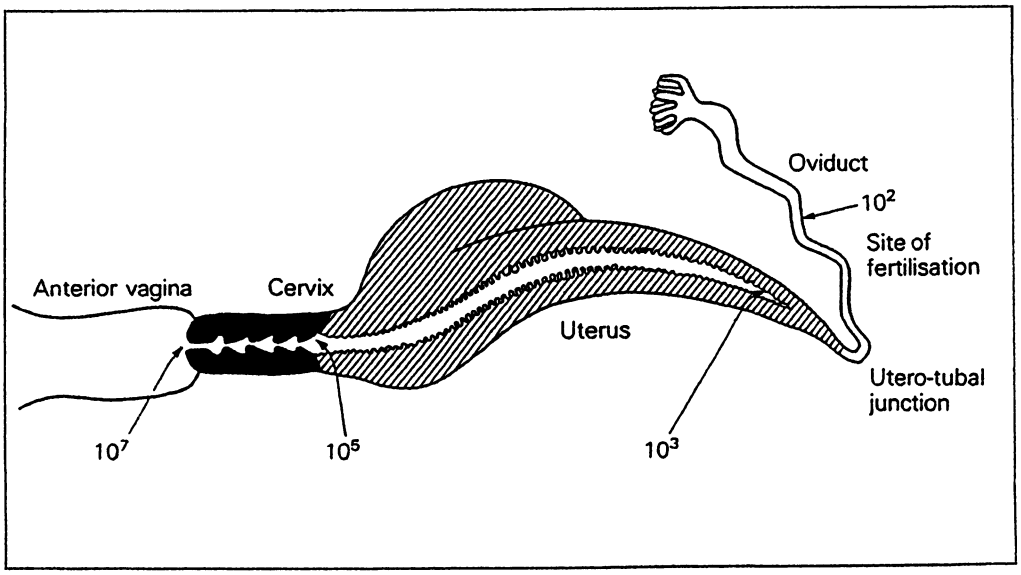

Figure 2. Diagrammatic representation of the genital tract of a cow to show the steeply-diminishing gradient in sperm numbers after mating between the site of ejaculation in the anterior vagina and the site of fertilisation in the Fallopian tube. At the time of activation of the newly-ovulated secondary oocyte, the sperm:egg ratio at the ampullary-isthmic junction may be close to unity.

rates) from individual bulls have seldom proved fully satisfactory. Future approaches to predicting fertility may need to devise a method for simulating the sequential influences of both male and female genital tract molecular constituents on the sperm suspension. In terms of a physiological perspective, it is worth bearing in mind what appears to be Nature's strategy: progressive post-testicular stabilisation of sperm cell membranes in the male duct system, especially during epididymal transit, followed by a progressive destabilisation in the female tract, especially within the Fallopian tubes. This would culminate in an acrosome reaction sensitive sperm at or close to the time of ovulation.

If the ultimate fertility of a sperm sample depends upon the product of such molecular interactions, then this should sound a note of caution to those laboratories pursuing 'male-only' prediction tests for fertility. At best, they may represent only half the picture and probably less when allowance is also made for egg factors at the site of fertilisation.

'Sex-separated' spermatozoa

A further reason for adopting the approach of deep uterine insemination would be in the context of predetermining the sex of an embryo using laser-optic distinguished X- and Y-chromosome bearing bull spermatozoa separated by flow cytometry (*Cran et al.* 1993). As developed by Johnson and his colleagues at USDA, Beltsville, and subsequently in association with workers in Cambridge, England (*Johnson et al.* 1989 & 1994, *Johnson* 1995), the technique of separating X- and Y-bearing spermatozoa is relatively slow and costly, leading to an overall limitation in the number of cells available for insemination. One means of overcoming this latter constraint is to introduce the sperm sus-

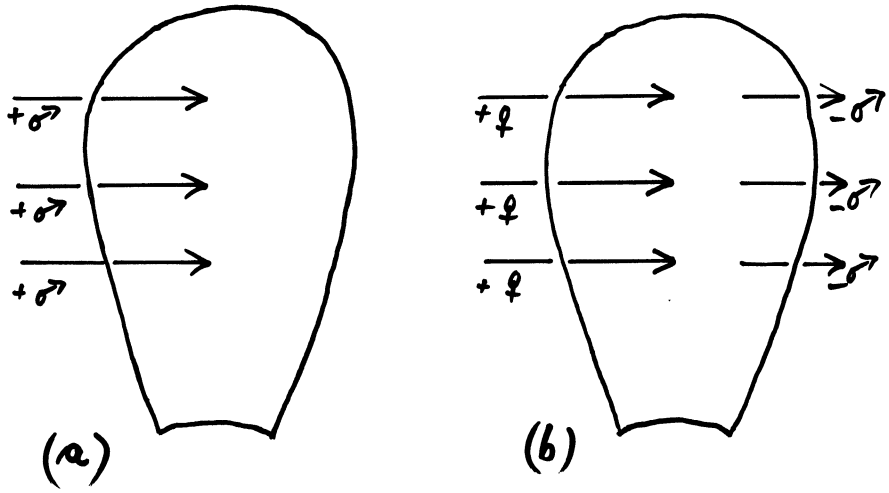

Figure 3. Diagrammatic representation of a sperm head to indicate:
(a) addition of stabilising factors (macromolecules) to the sperm plasmalemma during passage along the male duct system, especially during epididymal transit; and
(b) addition and/or substitution of further macromolecules on the sperm surface in the female duct system, in part in the uterine lumen but especially within the Fallopian tubes. These latter changes are thought to contribute to membrane remodelling as preparation for a coordinated acrosome reaction at or close to the egg surface (zona pellucida) shortly after the time of ovulation.

pension (*eg.* 1–2×10^5 spermatozoa in 0.1 ml) as far forward in the uterine horn as possible, close to the ipsilateral utero-tubal junction. This should increase the chances of an adequate number of viable spermatozoa being presented to the Fallopian tube, a proportion of which would – in due course – gradually become available close to the site of fertilisation.

As is invariably the case with a new or modified approach, there are aspects still to be adequately worked out concerning the most suitable volume of inseminate and optimum number of spermatozoa in the suspension. These parameters are currently being examined in at least one North American laboratory (*Seidel et al.* 1997 & 1998). Apart from the inherent fertility of the bull under test, they will probably depend on (a) the precise site of sperm deposition, and (b) on the interval elapsing before ovulation: ie. the longer the interval, the greater the number of sex-separated spermatozoa that might be required.

Traditional arguments against deep insemination

Palpation of pre-ovulatory ovary

Implicit in the proposal of deep uterine insemination is identification of the ovary bearing a pre-ovulatory follicle so that the uterine horn to be inseminated can be correctly manipulated. The traditional approach to such identification would be by means of ovarian palpation *per rectum* although, in an experimental or clinical laboratory situation, it could involve ultrasonic scanning. The potential hazard is that palpation of the ovaries of oestrous animals might lead to premature rupture of a pre-ovulatory follicle with expulsion of the oocyte and loss into the abdominal cavity. At least three points can be made in response.

1. Many experienced inseminators will readily admit to some exploratory palpation of the ovaries during their daily procedures, even though their guidelines expressly discourage this step. There is no persuasive evidence that careful exploration has a deleterious influence. Indeed, in circumstances in which the performance of inseminators is monitored, they themselves would quickly desist from ovarian palpation if it was found to be harmful.
2. There is no *a priori* reason to suppose that a clinician would be any more skilled at ovarian palpation than an experienced inseminator. The proportion of follicles ruptured in this manner in cows about to be inseminated is not known. A follicle on the verge of ovulation is becoming softer and eventually almost flaccid, and thus might be less susceptible to artificial collapse.
3. To the best of our knowledge, there is no specific evidence that premature rupture of a pre-ovulatory follicle leads to loss of the bovine oocyte into the abdominal cavity rather than capture by the Fallopian tube. Even in instances of premature artificial collapse with expulsion of the oocyte, the cilial activity of the fimbriated extremity and ampulla would act to draw the oocyte into the tubal ostium.

By contrast, in circumstances in which a follicle was not at the immediate pre-ovulatory stage and yet was artificially ruptured, it does not follow that the oocyte would inevitably be expelled. Its cumulus investment is anchored to the follicle wall as part of the *stratum granulosum* (see *Hyttel* 1988), and thus the oocyte might remain *in situ* after loss of fluid from the collapsed structure.

Perforation of uterine wall

A second reservation concerning deep insemination is the risk of damaging the mucosa or puncturing the wall of the uterus with the insemination device. Again, we know of no systematic study documenting the incidence of perforation although it does occur during conventional insemination. What is perhaps worth noting is that deep uterine passage of catheters introduced non-surgically via a luteal phase cervix (Fig. 4) has long been accepted for recovery of embryos (*Newcomb et al.* 1978, *Greve* 1981). Because bovine eggs commonly remain in the Fallopian tubes for 72–80 hours after ovulation (*Hamilton & Laing* 1946, *Betteridge* 1977), an important difference is that non-surgical embryo recovery from the uterus cannot be performed until at least three days after ovulation. The uterus would no longer be tonic. Deep insemination, on the other hand, would be performed into a tonic and thus 'shorter' uterus of an oestrous animal. A suitably-designed flexible catheter with rounded tip and side emission of the sperm suspension should more easily be guided around the horn of a shorter, supportive uterus towards the utero-tubal junction. A further modification to a round-tipped catheter could be a mobile roller tip to facilitate progress around the curvature under manual guidance.

A valuable aside is to consider a possible sequel if the wall of the uterus were to be inadvertently perforated and the sperm suspension deposited. In fact, this would correspond to intraperitoneal insemination, a technique first reported in cattle by *Skjerven* (1955) and *McDonald & Sampson* (1957), and since applied extensively with some success by *Lopez-Gatius* (1995) to overcome infertility in repeat breeder cows. Intraperitoneal insemination of oestrous cows seems not to lead to problems of infection, but fertility is generally poor unless insemination is performed shortly before ovulation. Why should this be so? Apart from dilution and dispersal of the sperm suspension in the abdominal cavity, a functional sperm reservoir would not be estab-

lished in the caudal isthmus of the Fallopian tube, so an accurate timing of insemination shortly before ovulation would be essential. Even so, perforation of the uterine wall could lead to introduction of the sperm suspension in the vicinity of the ovaries, so fertilisation might be a sequel to this exceptional event. A more serious aspect would concern the size of the puncture wound. This would depend on the size and form of the insemination device, and whether eversion of the mucosa and/or haemorrhage followed damage to the uterine wall.

Risk of polyspermic fertilisation

One consequence of deep insemination, even with a reduced number of spermatozoa, might be to increase the incidence of penetration of the egg vitellus by more than one spermatozoon, that is polyspermic fertilisation. However, the limited evidence available to date suggests that this risk is low, for the polyspermic condition has seldom been recorded in cow eggs fertilised *in vivo.* If correct, this would seem to be because the bovine utero-tubal junction and caudal isthmus act powerfully to control the number of competent spermatozoa ascending to the site of fertilisation, not least by means of the 'sperm trapping' activity of conspicuous glands close to the utero-tubal junction (*Hunter* 1995). There is the related aspect that the bovine embryo appears to have a robust and long-lasting block to polyspermy *in vitro,* even in the face of overwhelming numbers of freshly capacitated spermatozoa (*Hunter et al.* 1998). Nonetheless, because the proposed site of insemination would be close to the utero-tubal junction and spermatozoa would be initially suspended in a fluid other than uterine fluid, these modifications might together prompt changes in the steep sperm gradient that prevents polyspermy in the physiological situation.

The risk of polyspermic fertilisation after procedures of deep insemination certainly needs to be clarified, not least because the condition is pathological in mammals, invariably leading to pre-implantation death of the embryo and usually its early loss (*Beatty* 1957, *Piko* 1961, *Austin* 1963). We intend to conduct experimental studies at the Danish Institute of Animal Science, Foulum, in the summer of 1998 to examine the influence of depositing excessive populations of fresh or frozen-thawed bull spermatozoa in the tip of the uterine horn. Eggs would be recovered within 24 h of spontaneous ovulation to quantify the incidence of this anomaly of fertilisation. The low numbers of accessory spermatozoa usually noted on or in

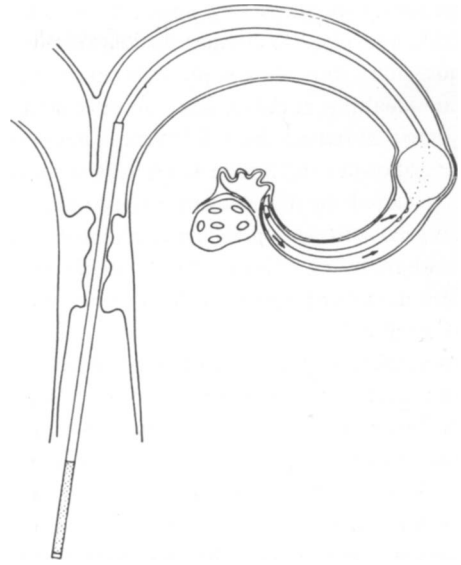

Figure 4. Diagrammatic representation of passage of a catheter through the cervix and around a uterine horn (under manual guidance) during non-surgical recovery of embryos from the uterine tip. A comparable non-surgical passage of an insemination catheter (without, of course, an inflatable cuff) around a tonic uterine horn should cause no greater difficulty. This would be especially so if there were a rounded or mobile (roller) tip to the catheter and side emission of the sperm suspension.

the zona pellucida of cow eggs (*Laing* 1945 & 1957, *Greve* 1981) compared with the 200 or more zona spermatozoa seen, for example, in individual pig eggs, suggest sperm regulating systems of remarkable effectiveness in the upper regions of the bovine tract. This is further emphasised by the paucity of spermatozoa recorded in the Fallopian tube ampulla of mated or inseminated cows (*Thibault* 1973, *Hyttel* 1988, *Larsson* 1988).

As to whether deep insemination would increase the risk of fertilisation by abnormal spermatozoa, there is no evidence that this would be so. Procedures of *in vitro* fertilisation that expose secondary oocytes to dense suspensions of motile spermatozoa seem not to promote an elevated penetration by morphologically abnormal spermatozoa. And, at least under *in vivo* conditions, there is evidence from other mammals (*eg.* rabbit: *Mortimer* 1978; cow: *Larsson* 1988) that a form of selection takes place: spermatozoa reaching the site of fertilisation have selected themselves for appropriate motility, morphology and surface characteristics, and the converse situation also applies. Even with deep insemination, the region of the utero-tubal junction and full extent of the isthmus would still intervene between the site of sperm deposition and the site of fertilisation.

Professional inseminators versus clinicians

One of the more sensitive aspects of this essay concerns the relationship between qualified clinicians and artificial inseminators. The latter constitute a body of highly skilled technicians whose competence can be monitored, at least in part, by their success rates (non-return rates) for individual bulls across large numbers of cows. No comparable monitoring is customary for their veterinary clinical colleagues, although performance data are beginning to feature quite widely in the medical, especially surgical, profession. The point here is that bovine insemination deep into a uterine horn is invariably seen as a specialist clinical procedure whose application should be strictly limited to the veterinary profession. Can such a view be maintained in 1998 when the primary objective is progress? If it is accepted that inseminators are manipulating the reproductive tract of cows and heifers *per rectum* on a daily basis and indeed many times per day, and that most inseminators are employed on a mid-to-long term basis, then it should be clear that they will be at least as competent as a qualified veterinarian. They invariably have a full grasp of the relevant anatomy, if not of all the Latin names, they recognise and understand the significance of tonic reproductive tissues, and they are quick to distinguish morphological anomalies of the genital tract. After further specialist training for the deeper inseminations proposed, and with some emphasis placed on the potential hazards of the technique, they should be well prepared to achieve the modified site of sperm deposition. To put this into a practical perspective, it is suggested that a comparison of the results from 100 inseminators using the new procedure with those from 100 clinicians would not be unfavourable to the former.

Conclusion

As a final remark, the present proposals appear to be reasonable, straightforward and well worth testing extensively. The results of *Lopez-Gatius & Camon-Urgel* (1988), *Senger et al.* (1988) and *Seidel et al.* (1997, 1998) employing cornual insemination in meaningful numbers of cows already offer encouragement. The artificial insemination industry might receive a welcome boost from this modified approach, one which could clearly have relevance for other bovine and non-bovine ruminant species. This would be especially so should sperm numbers available become a limiting factor or if frozen-

thawed spermatozoa of certain bulls proved unduly sensitive to the lower reaches of the female tract.

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Sammendrag

Dyb uterin inseminering hos kvæg. En frugtbar fremgangsmåde med færre spermier.

I nærværende oversigtsartikel har vi indledningsvis beskrevet forhold omkring befrugtningssted og etablering af det funktionelle spermiereservoir i de hunlige kønsorganer. Dernæst beskrives en modificeret præovulatorisk insemineringsteknik, hvor en lavere dosis sædceller deponeres dybt i uterushornet ipsilateralt til det ovarium, som indeholder den præovulatoriske follikel med henblik på at skabe et tilstrækkelig stort reservoir i isthmus. I forbindelse med ovulation frigøres og aktiveres sædcellerne fra dette depot.

Potentielle fordele ved dyb intrauterin insemination ville være: forbedring af fertiliteten for visse genetisk værdifulde tyre, hvis omløberprocenter er for høje, reduktion af insemingsdosis og anvendelse af kønssorteret sæd.

Mulige ulemper kunne være palpation af ovariet umiddelbart inden ovulation, perforation af uterus væggen eller blot beskadigelser af slimhinden og risiko for en øget hyppighed af polyspermi. Disse forhold er diskuteret, og der synes god grund til, at man indleder praktiske forsøg med denne insemineringsteknik.

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