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**Mother child bonding at one year; associations with symptoms of postnatal depression and bonding in the first few weeks**

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## **Abstract**

### **Purpose**

Some mothers experience neutral or negative feelings toward their new infant. This study examined the association between symptoms of postnatal depression and mother-infant bonding and the persistence of these feelings over the first year.

### **Methods**

Bonding was assessed using the Mother Infant Bonding Scale (MIBQ), at 4 times postnatal, “early weeks” (1 – 4 weeks), 9 weeks, 16 weeks and one year, in 50 depressed, Edinburgh Postnatal Depression scale (EPDS)  $\geq 13$  at 4 weeks postnatal, and 29 non depressed mothers.

### **Results**

A significant association between the EPDS score at 4 weeks and bonding score at 1 - 4 weeks, 9 weeks, and at 1 year postnatal,  $X^2(1) = 9.85, p < 0.01, 5.44, p < 0.05$  and  $5.21, p < 0.05$  respectively was found, with a trend at 16 weeks. There was a strong association between bonding in the early weeks and all later time points  $X^2(1) = 17.26, p < 0.001, 7.89, p < 0.01$  and  $13.69, p < 0.001$  respectively. Regression showed early bonding rather than early depression was the major predictor of bonding at one year.

### **Conclusion**

Women who are depressed postnatally can fail to bond well with their baby and this can persist for a year. Early identification and intervention for poor bonding is indicated.

*Key words:* postnatal, depression, bonding, mother, infant

## **Introduction**

A mother's thoughts about her baby stimulate affection and protective feelings, which facilitate the beginning of the mother-infant relationship (Ainsworth and Bell 1974). These feelings (Kumar, 1997) usually begin during the pregnancy. The mother's feelings about her baby, described as bonding, normally continue and increase when the baby is born, and underpin the development of her relationship with her child. Robson and Kumar (Robson and Kumar 1980) showed that up to 40% primiparae and 25% of multiparae mothers were indifferent to their babies on first holding them, although most developed affection within the first week.

The mother's feelings toward her unborn infant have also been described as maternal attachment (Cranley 1981) or prenatal attachment (Muller 1993). Attachment was first used to describe a two-way relationship in which both parties are active (Bowlby 1969). Cranley's Maternal-Fetal Attachment Scale includes a subscale called "Interaction" in which the mother can record physical actions by the unborn child that she perceives to be the response to her own actions. In contrast mother – infant bonding describes the mother's feelings towards her infant that do not require a response of any kind from the child. While both concepts are of interest, it is the mother-infant bonding that this study focuses on.

For some mothers affectionate feelings towards their infant fail to develop. This failure has been described as a disorder of mother-to-infant bonding, or more simply, bonding disorder (Kumar 1997; Brockington, Oates et al. 2001). A failure to bond with the infant may lead to rejection or harm in extreme cases. Brockington reports 10 – 25% of women referred to psychiatrists after childbirth experience bonding disorder (Brockington 1996). Further research showed that outright

rejection of the child is only found in 0.5 -1.0% of cases (Brockington, Aucamp et al. 2006).

There can also be variations in bonding in the general population. A study by Figueiredo and co-workers (Figueiredo, Costa et al. 2007) distinguished between strong bonding (66.6%) moderate bonding (29.4%) and no bonding (4%) within the first 24 hours postnatal. Two prospective studies have reported rates of poor bonding in the general population of postnatal women to be 7.1% at two weeks postnatal (Reck, Klier et al. 2006) to 8.9% at 12 weeks (Taylor, Atkins et al. 2005). Generally the earlier the measure the greater the percentage of poor bonding found. Recently Bienfait and co-workers (Bienfait, Maury et al. 2011) found 12.2% of women at 48 hours postnatal scoring over the threshold for high risk of poor bonding on the mother-infant bonding scale (MIBQ $\geq$ 2). The general improvement in bonding scores with time agrees with the earlier observations by Robson and Kumar (1980), however the persistence of bonding problems for a minority of mother infant dyads remains an important issue.

Some studies have noted an association between postnatal depression and poor bonding. Kumar (1997) showed that women who were suffering from postnatal depression had prolonged difficulties in developing maternal feelings towards their infants compared to women who were not depressed. In a previous study our group found a correlation between early postnatal symptoms of depression (EPDS  $\geq$ 13 on day 3 postnatal) and worse bonding scores within the first 12 weeks postnatal (Taylor et al. 2005). A recent Japanese study has also found an association between postnatal depression and poorer bonding (Yoshida, Yamashita et al. 2012). Women with postnatal depression do not all experience problems with bonding, and some women, who are not depressed, do experience poor bonding (Righetti-

Veltema, Conne-Perreard et al. 2002; Klier, Schmid-Siegel et al. 2006). Further research is needed to establish the extent to which postnatal depression and poor mother-infant bonding are associated.

The current study was designed to examine the persistence of poor bonding over the first year and association between symptoms of postnatal depression and mother-infant bonding.

## Methods

### Participants and procedure

This work was designed as a prospective cohort study. Hammersmith Hospital NHS Ethics Committee granted ethics approval. Women were recruited on the postnatal ward of Queen Charlottes and Chelsea Hospital, London (Figure 1). Participants were given verbal and written descriptions of the study and written consent was obtained. Only those mothers with healthy, full term (over 36 weeks) babies were approached. Women were also excluded from the study if they had a recorded history of a psychotic disorder, were under 17 years of age, did not speak sufficient English or were receiving support from social workers for housing or social problems. Those who were willing to take part were screened for depressive symptoms at four weeks postnatal, sent to them by post. Of the 2048 women who returned their questionnaires 285 women (13.9%) met screening criteria for high risk of depression (EPDS  $\geq$  13). They were invited to take part in the study group, and 89 women agreed to participate, of whom 50 completed the study. This group is presented here as the depressed group. Of the women whose screening scores indicated they were not depressed, 1376 (67.2%) scored EPDS  $\leq$  8, 95 were selected at random and invited to take part in the study. This group is described as the non-depressed group. Forty five of these women agreed to participate and 29 completed the study. Women who scored from 9 to 12 on the EPDS at screening were not invited to take part in the study, to clearly delineate the depressed and non-depressed. All the women were asked to complete the depressive symptoms questionnaire and mother – infant bonding questionnaire at 9 weeks, 16 weeks and one year postnatal. At 9 weeks postnatal the women were given two versions of the bonding questionnaire,

the first asked the women to describe her feelings in “the early weeks” (1 – 4 weeks postnatal) and the second asked for her current feelings.

As part of a larger study, the depressed group were randomly assigned either to a support group or to a baby massage group for six weeks between week 9 and 16 postnatal, as described in O’Higgins et al (2008). The intervention study was designed to investigate possible effects of attending a baby massage group in the early postnatal period compared with attending a general support group, on maternal mood, mother-infant interaction and infant behaviour. There were no differences in bonding scores at any time point between the massage and the support groups, so they were combined here for this analysis (O’Higgins et al 2008).

## **Measures**

### Depressive Symptoms

The EPDS (Cox et al. 1987) is a ten item self report questionnaire developed for use in the early postnatal period as a screening tool to enable primary healthcare workers to identify women who may need services for postnatal depression. The 10 item EPDS was validated by Cox and co-workers by comparison with clinical diagnosis using the Research Diagnostic Criteria (RDC) (Spitzer, Endicott et al. 1975). A threshold of 12/13 was found to include all those women with an RDC diagnosis of Definite Major Depressive Illness (Cox et al 1987). However, Cox and co-workers also suggested that a score of 9 or 10 may be an appropriate threshold for routine use by primary health care workers identifying mothers who may need treatment for depressive symptoms (Cox, Chapman et al. 1996). In this study a score of 13 or more on the EPDS was used. The Cronbach alpha was 0.78.



Women for the non-depressed group were randomly selected from those who returned an EPDS score between 1 and 8. The upper limit of 8 was chosen, to avoid including women in the control group who might be found to be mildly depressed.

### Bonding Questionnaire

The MIBQ (Taylor et al., 2005), is an 8 item self-rating scale. Each item describes an emotion the mother may experience towards her infant and is a statement which is rated on a 4-point Likert scale. The range for the total score is 0 - 24. Positive and negative emotions are scored in the opposite way. Higher total scores indicate worse bonding. The Cronbach alpha for the bonding scale was 0.71. A score of 2 or more on the MIBQ has been shown to indicate poor bonding (Bienfait et al, 2011) and was used as the cut-off in this study.

### Statistical analyses

The EPDS data were normally distributed, but bonding data were not and could not be normalised by transformation. Non parametric tests were therefore used. Spearman rank correlation was used to compare the bonding scores at each time point.  $X^2$  was used to test the association between psychometric rating scale data at different time points. A score of 13 or more on the EPDS was used as the criterion for inclusion in the depressed group (Cox et al, 1987). A score of 2 or more on the MIBQ was used to indicate poor bonding (Bienfait et al, 2011).  $X^2$  was used to compare the demographic data of the women in the depressed and non depressed groups. Where the expected number in any cell fell below 5 Fisher's Exact was used. Mann-Whitney U tests were used to compare the bonding scores

for the depressed and the non-depressed groups. Logistic regression was used to test the putative predictors of poor bonding at one year.

## **Results**

Results are reported for the 79 mothers who completed the study, 50 in the depressed group and 29 in the non-depressed group. The characteristics of the participants are given in table 1. There were no differences in demographic data between those who completed the study and those who started, but failed to complete the study. Reasons for not completing included moving out of the area and returning to work full-time.

The demographic data for those women who completed the screening data collection but declined the offer to take part in the study showed they had a similar profile to the participants. The women who declined had a mean age of 32.5 (SD:4.6); educational status (UK educational stages: General Certificate of Secondary Education (GCSE) at 16 years old; Advanced Level (A'Level) at 18 years old) (Pre GCSE 0%, GCSE 10%, A'Level 20%, university 55% and vocational qualification 15%); ethnicity (80% white, 10% black, 10% other); nationality (55% UK born) and marital status (95% married or living together, 5% not living together). The ethno-cultural origins of the women who completed the study and those who declined to take part are both typical of the women who attend Queen Charlotte's Hospital. The catchment area of the hospital is in west London and is multi-racial, but generally fairly affluent. Information about the baby born immediately prior to the woman taking part in this study (index child) and siblings showed no significant differences in the depressed and control groups with regard to parity (primiparous 70%, 1 or 2 other children 25%, 3 or more 5%), and breast feeding (76.5%), except

in the sex of the index child (40% male in the depressed group and 69% male in the non depressed group,  $p=0.013$ ). There was no difference in the proportion of women who scored EPDS  $\geq 13$  in the non participant group compared with those who did participate, which indicates that there was no bias created by the loss of these potential participants.

In general the whole cohort was well educated and married or living with the father of the child (Table 1). In the non-depressed group of women there was a trend towards higher education and a greater proportion born in the UK, but neither trend reached statistical significance. There was a difference between groups with the sex of the baby. More of the non-depressed women had boys than girls  $X^2(1) = 6.16$ ,  $p=0.019$ . There were no other significant differences between the two groups. Logistic regression showed that none of these factors (the sex of the child, being born in the UK and educational status of the mother) predicted bonding outcome at one year.

*Mother-infant bonding scores compared over time.*

There was a strong correlation between bonding in the early weeks (1-4 weeks) and 9 weeks, 16 weeks and one year postnatal (figure 1a,b,c). The Spearman Rank Correlation was found to be  $R_s=0.62$ ,  $p<0.001$ ;  $R_s =0.59$ ,  $p<0.001$  and  $R_s =0.50$ ,  $p<0.001$  (2-tailed) respectively.

If a woman experienced poor bonding ( $MIBQ \geq 2$ ) in weeks 1 - 4 postnatal, she was 15.75 times more likely to still be having problems with bonding one year postnatal ( $X^2(1) = 17.26$ ,  $p<0.001$ ). The bonding scores at 9 weeks and 16 weeks postnatal also predicted bonding at one year. Women with poor bonding at 9 weeks postnatal were 4.19 times more likely to have poor bonding at one year  $X^2(1) = 7.89$ ,

$p < 0.01$ , and women with poor bonding at 16 weeks were 7.48 times more likely to have poor bonding at one year postnatal  $X^2(1) = 13.69$ ,  $p < 0.001$ .

*EPDS scores and mother-infant bonding scores diagnosis as predictors of later mother-infant bonding.*

The EPDS scores at 4 weeks postnatal were then used to define the depressed group (EPDS  $\geq 13$ ) from the non depressed group for the rest of the analysis. There was a significant difference in mean EPDS scores between the depressed group and the non-depressed group of women at each time-point (Table 2). Although the scores of the depressed group improved over the year they remained significantly higher than the non depressed group. Comparison of the bonding scores for the depressed and the non-depressed groups (defined by the EPDS score at 4 weeks) by Mann-Whitney U test showed a difference between them in the early weeks (1-4 weeks),  $p < 0.001$  at 9 weeks  $p = 0.001$ , at 16 weeks  $P < 0.5$  and at 1 year postnatal  $p < 0.05$  (Table 2).

The proportion of depressed and non-depressed women who also experienced poor bonding was examined. If a woman scored  $\geq 13$  on the EPDS at week 4 she was 5.13 times more likely to be experiencing poor bonding (MIBQ  $\geq 2$ ) at the same time ( $X^2(1) = 9.85$ ,  $p < 0.01$ .) At one year postnatal 24.1% of women from the depressed group (EPDS  $\geq 13$  at 4 weeks) scored 2 or more on the MIBQ, while only 5.1% of the non-depressed women scored did so ( $X^2(1) = 5.21$ ,  $p < 0.05$ ).

Concurrent EPDS data were compared with the bonding data at one year and showed that 14 women scored EPDS  $\geq 13$  and 22 women scored MIBQ  $\geq 2$ , but only 6 of these women scored both. The  $X^2$  test revealed that there was no association between the EPDS and MIBQ scores at this time point.

Both EPDS at 4 weeks and bonding in the early weeks were associated with bonding at one year. However when both factors were entered simultaneously into a logistic regression, it was only the early bonding scores that predicted bonding at one year  $B(SE)=2.62(0.92)$ , Wald =8.14,  $p<0.01$ .

## **Discussion**

There was a strong association between scores on the MIBQ in the early weeks postnatal and the bonding scores at all other time-points (Figure 1). There was also a strong association between the EPDS scores at 4 weeks postnatal and the bonding scores at one year. However, logistic regression showed that early bonding, rather than early depression, was the main predictor of bonding at one year.

The study, depressed, group in this analysis comprises two intervention groups; baby massage classes or support group. There were no differences in bonding scores at any time point between the women who attended baby massage classes and the women who attended a support group (O'Higgins et al 2008). A no-treatment depressed-group was not included in the study for ethical reasons. For this study these two intervention groups were combined for further analysis.

It might be argued that poor bonding is only one aspect of postnatal depression and therefore current screening for postnatal depression is sufficient to identify those mothers in need of support services. Studies that report a link between maternal depression and poor mother-infant bonding (Kumar 1997; Clark, Tluczek et al. 2003; Taylor, Atkins et al. 2005; Moehler, Brunner et al. 2006; Figueiredo, Costa et al. 2007; Figueiredo, Costa et al. 2009), support this. On the other hand some studies distinguish between maternal depression and bonding

disorder (Fleming, Ruble et al. 1988; Klier 2006; Brockington 2008). The results presented here suggest that although there is an association between postnatal depression and poor bonding in the early weeks  $X^2(1) = 9.85, p < 0.01$ , the early bonding score is the strongest predictor of later bonding, and so it is important to examine bonding in its own right.

These disorders may share common triggers or one may influence the development of the other. Hoffman and Drotar reported results that suggest maternal depression may have selective effects on different aspects of mother-infant interaction (Hoffman and Drotar 1991). They found that women suffering from depressive symptoms showed less positive interaction overall, with reduction in expressivity / affective involvement and responsivity / sensitivity, while the level of maternal stimulation and infant activity were not different from non-depressed controls. A prospective study by Roisman and co-workers (2002), showed an association between the mother's attachment style and symptoms of depression further suggesting a common underlying process might be identified.

Bonding disorders can also occur in the absence of postnatal depression (Brockington 2004). Klier reported specific case studies to illustrate her view that bonding disorder is distinct from postnatal depression (Klier 2006). In the first case Klier notes that while the patient's EPDS scores fell below the threshold for depression by week 4 postnatal, her bonding scores remained above the threshold for poor bonding until week 14. In the second case the patient recovered from postnatal depression but continued to have poor bonding with her infant daughter.

In a prospective study of new mothers, Fleming and co-workers (1988) used three items to describe the mother's feelings about her infant (e.g. *Thinking of baby makes me feel good; Talk a lot about baby*). While they found that maternal mood

did influence two of the study outcomes (*maternal adequacy feelings; attitudes to caretaking*), they found that neither antenatal nor postnatal mood scores made significant contributions to the variance of maternal feelings about her infant (Fleming, Ruble et al. 1988). There is evidence that when the infant has become an active player in the mother-infant interaction there is a link between maternal depression and less effective mother-infant interaction (Feldman 2007), but there are also mother-infant dyads who experience poor interaction in the absence of maternal depression. This was illustrated by a prospective study by Rigetti-Veltma and co-workers (2002) that recruited 570 unselected pregnant women. To assess the mother-infant relationship at 3 months Rigetti-Veltma and co-workers used two instruments (Guaraldi, Caffo et al. 1985; Bur, Gozlan et al. 1989). The scale by Bur and co-workers (1989) is an observer rated scale describing aspects of the mother-infant interaction. The latter is a two part scale administered by trained midwives; the first part is answered by the mother and includes her feelings about the child, while the second part is answered by the midwife describing the mother-infant interaction. Rigetti-Veltma and co-workers found 10.6% of the non-depressed women had a global interaction with their infant measured on the scale by Bur and co-workers as pathological and 11.5% of infants of non-depressed women showed a poor relationship with their mother on the scale by Guaraldi and co-workers (Rigetti-Veltma, Conne-Perreard et al. 2002), thus providing evidence for cases of poor interaction in the absence of maternal depression.

In a study using video analysis of the interaction between mothers and their infants at 2 months old, Murray and co-workers (1996), showed that depressed mothers were more likely to have problems interacting with their babies (Murray, Fiori-Cowley et al. 1996). However, it was the quality of the interaction at 2 months,

not the depression score that predicted the cognitive function of the child at 5 years. It is interesting to note that a recent study by Tharner and co-workers (2012) into maternal depression and infant –mother attachment found that maternal depressive disorder, regardless of severity or psychiatric co-morbidity, was not associated with an increased risk of infant attachment insecurity or disorganisation.

Brockington (2008) points out that intervention methods usually involving directed play are effective for bonding disorder. A randomised control trial of 117 mothers showed that 8 weeks of interaction coaching significantly improved mother-infant interaction compared to the control group (Horowitz, Bell et al. 2001). A review by (Poobalan, Aucott et al. 2007) examined the effects of treating PND on mother-infant interaction and child development. Treatments involving mother and child were found to improve the mother's feelings about her child, while treatments involving the mother alone were found to be effective for depression but did not have a significant effect on the mother's feelings towards her child. This indicates that early detection and referral to appropriate services could provide effective treatment.

The data presented in this study show a strong association between early depressive symptoms as measured by the EPDS (weeks 1- 4) and poor bonding scores on the MIBQ. This situation changed by one year postnatal when there was no longer an association between depressive symptoms and poor bonding. For some women the bonding score remained poor while the depressive symptoms improved and for others the reverse was true. This suggests that while these two disorders may share common triggers they may follow different courses of development or resolution. Research into the underlying neuro-endocrinology of bonding (Douglas 2010), and postnatal depression (Bloch, Daly et al. 2003), may help to explain these differences.



While it is clear that postnatal depression is associated with a risk of poorer cognitive and behavioural development of the child, (Murray 1992; Sharp, Hay et al. 1995; Hay, Pawlby et al. 2001; Pawlby, Sharp et al. 2008), it has not been established to what extent poor bonding contributes to this. Earlier studies of postnatal depression and child development did not measure mother infant bonding. Future long-term studies should distinguish different sub-groups: women who are depressed and experiencing poor bonding; women who are depressed but not experiencing bonding problems and women who are not depressed but experiencing bonding problems. It is also important to establish the predictive value of bonding disorder for later attachment problems. This will establish the impact of each factor on the cognitive and behavioural development of the child born at the time mother is affected.

The study presented here is a prospective study of mother-infant bonding up to one year postnatal. However, there are some limitations due to the study being part of a larger overall project. The study group comprises mother and infant pairs who were assigned randomly to two different treatment conditions, one being mother-baby massage and the other attendance of a support group (O'Higgins, St James Roberts et al. 2008). These were found to have no different effect on outcome which enabled their combination for this study. Another limitation of the current study is the discontinuous data groups created by selecting women with particular scores on the EPDS for the study and control groups. This restricted the choice of statistical analysis. Both limitations could be avoided in the design of future projects. It would also have been of interest to study the mother's own attachment style, which might in turn have been related to impaired bonding.

In conclusion, screening for postnatal depression, by administration of the self report EPDS scale (Cox, Holden et al. 1987) or questions presented verbally by primary healthcare workers (NICE 2007), has become part of normal practice in developed countries, and widely reviewed (Delatte, Cao et al. 2009; Hewitt and Gilbody 2009; Mitchell and Coyne 2009). The results presented in this paper suggest that a specific screening for risk of poor bonding may also be important although more research will be needed to evaluate potential benefit versus harm. In the future, appropriate referral services may play a role in preventing child rejection or harm.

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## Legend to Figure 2.

Figure 2. Bonding data for all participants comparing scores in the early weeks (1-4 weeks postnatal) with bonding at 9 weeks (2a); bonding at 16 weeks (2b); bonding at 1 year (2c) and regression line for each, Spearman Rank Correlation  $S_r=0.62$ ,  $p<0.001$ ;  $S_r=0.59$ ,  $p<0.001$  and  $S_r=0.50$ ,  $p<0.001$  (2-tailed) respectively.

**Table 1. Demographic data**

	Depressed EPDS≥13	Non- depressed	P
	N=50	N=29	
Maternal Age mean (SD)	32.3 (5.3)	34.6 (4.9)	NS
Education: Pre GCSE %	4.0	0	NS
GCSE %	8.0	3.6	
A' Levels %	20.0	21.4	
University %	50.0	71.4	
Vocational %	18.0	3.6	
Ethnicity: White %	70.0	86.2	NS
Black %	14.0	13.8	
Asian %	10.0	0	
Other %	6.0	0	
UK born: % yes	50.0	72.4	NS
Marital status:			NS
Married/Living together %	86.0	96.6	
Not living together %	14.0	3.4	
Parity: Primiparous %	70.0	86.2	NS
1 or 2 other children %	28.0	13.8	
3 or more others %	2.0	0	
Sex of index child: % male	40.0	69.0	P=0.013
Breast Feeding: %	74.3	82.2	NS

Demographic data of the depressed and non-depressed group was compared by  $X^2$  except where the expected number in any cell fell below 5, and Fisher's Exact was used instead. NS=no significant difference The index child is the baby born immediately prior to the mother participating in the study. Education is defined by the UK system: General Certificate of Secondary Education (GCSE) at 16 years old; Advanced level exams (A'Levels) at 18 years old. School pupils who leave at the age of 16, without any exam qualification are included as Pre GCSE. Vocational training is an alternative to higher education at university.

**Table 2. Psychometric rating scale data for the depressed and non-depressed group compared at each time-point.**

	Depressed EPDS≥13	Non- depressed	P
	N=50	N=29	
EPDS 1-4 weeks median (range) mean (SD)	17.0 (13-22) 16.7 (2.6)	5.0 (1-8) 4.9 (1.9)	P<0.001
EPDS 9 weeks median (range) mean (SD)	13.0 (7-22) 13.5 (4.0)	3.0 (0-10) 3.1 (2.7)	P<0.001
EPDS 16 weeks median (range) mean (SD)	10.0 (0-26) 10.0 (5.0)	3.0 (0-10) 3.7 (2.4)	P<0.001
EPDS one year median (range) mean (SD)	8.5 (1-27) 9.5 (5.7)	3.0 (0-16) 3.4 (3.2)	P<0.001
Bonding Scores 1-4 weeks median (range) mean (SD)	3.0 (0-19) 4.8 (4.9)	1.0 (0-4) 1.3 (1.3)	P<0.001
Bonding Scores 9 weeks median (range) mean (SD)	3.0 (0-15) 2.4 (2.9)	0 (0-3) 0.8 (1.1)	P<0.01
Bonding Scores 16 weeks median (range) mean (SD)	1.0 (0-10) 1.6 (2.5)	0 (0-3) 0.6 (0.9)	P<0.05
Bonding Scores 1 year median (range) mean (SD)	1.0 (0-9) 1.6 (2.0)	0 (0-6) 0.7 (1.3)	P<0.05

Mann Whitney U test used to compare psychometric rating scale data for the depressed and non-depressed group at each time-point.



**Figure 1. Study Design**

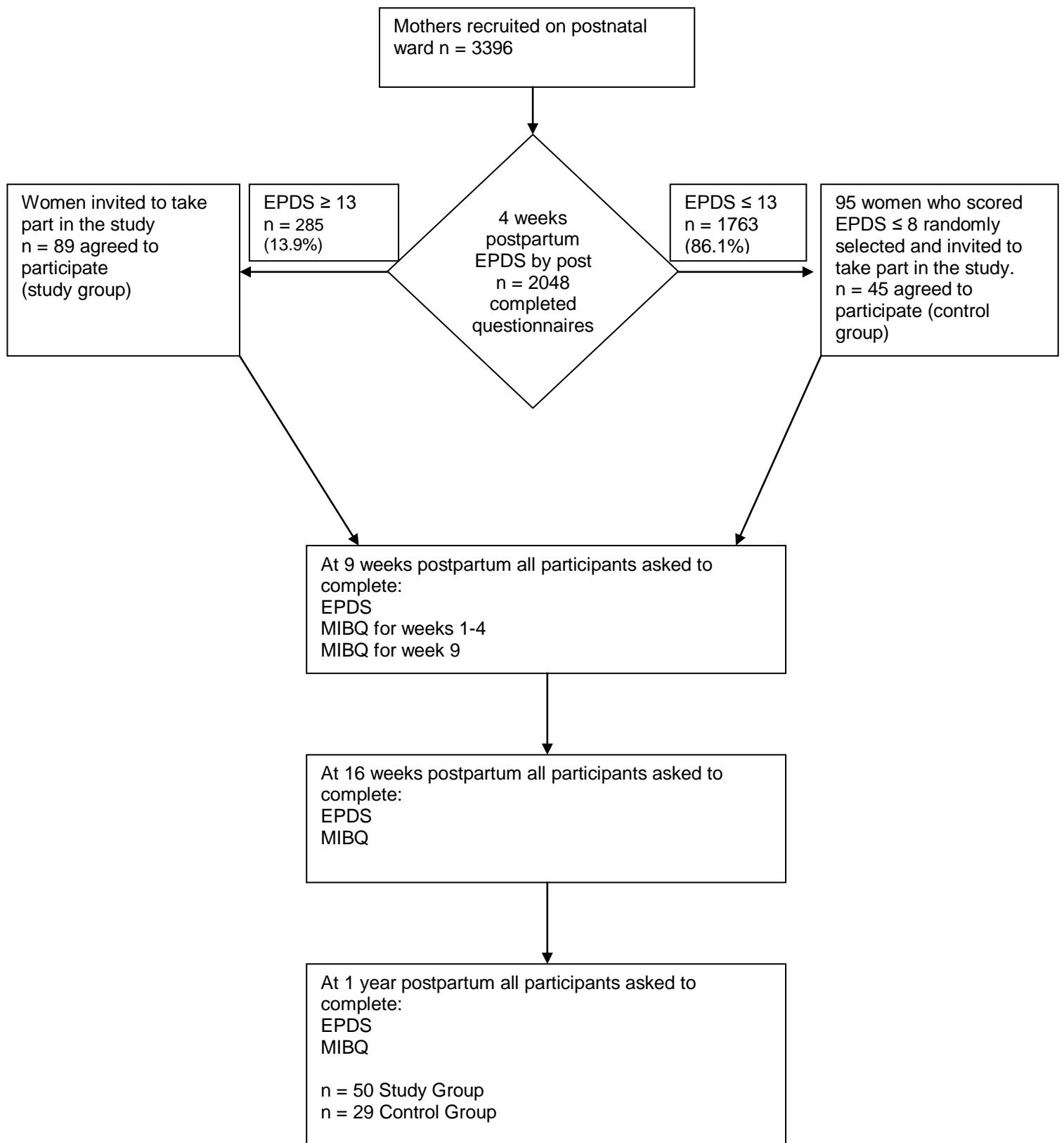


Figure 2.

